Accumulation of triacylglycerol in cultured rat hepatocytes is increased by ethanol and by insulin and dexamethasone

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Isolated hepatocytes from female rats were cultured in HI-WO/BA medium for 6 days. To the medium was added oleate, ethanol, dexamethasone and insulin. With oleate alone, triacylglycerol accumulated; ethanol augmented the accumulation by 90%. To the best of our knowledge, this is the first demonstration that ethanol *in vitro* increases the content of triacylglycerol in liver cells. Further addition of dexamethasone or insulin did not alter the accumulation of triacylglycerol, indicating that these hormones did not play permissive roles for the effect of ethanol in the present system. Dexamethasone and insulin, in the absence of ethanol, increased the accumulation of triacylglycerol by 30% and 50% respectively. The concentration of glycerol 3-phosphate was increased in the presence of ethanol; however, with time the concentration of glycerol 3-phosphate declined almost to control values, while the accumulation of triacylglycerol continued linearly; this suggests that the effect of ethanol was not mediated via fluctuations in the concentration of glycerol 3-phosphate. These results are discussed in relation to earlier investigations *in vivo* and *in vitro*.

During prolonged ethanol ingestion, triacylglycerol accumulates in cytoplasmic lipid droplets of rat liver parenchymal cells (Iseri *et al.*, 1966; Kondrup *et al.*, 1979b). In perfused rat liver, ethanol did not increase the accumulation of triacylglycerol in cytoplasmic lipid droplets; the hepatic content of diacylglycerol, however, was doubled in the presence of ethanol (Kondrup *et al.*, 1979a). Other workers (Fellenius *et al.*, 1973; Wirthenson *et al.*, 1980) reported increases in triacylglycerol in the presence of ethanol in experiments with perfused liver and isolated hepatocytes; however, these workers did not distinguish between di- and tri-acylglycerol, and the increase in total acylglycerol was similar to the increase in diacylglycerol mentioned above. It therefore remains to be demonstrated *in vitro* that ethanol increases the hepatic accumulation of triacylglycerol. Experiments with hepatocytes in culture were undertaken in the present study, since longer periods of exposure to ethanol may be required for ethanol to elicit its effect on hepatic metabolism of triacylglycerol.

In vivo, glucocorticoids play a role for the accumulation of triacylglycerol after a single dose of ethanol (Maickel & Brodie, 1963), partly perhaps by increasing the activity of hepatic phosphatidate

phosphohydrolase (Pritchard *et al.*, 1977, 1979). Insulin antagonizes the effect of glucocorticoids on phosphatidate phosphohydrolase in isolated hepatocytes (Lawson *et al.*, 1982). Therefore experiments with dexamethasone and insulin were also included in the present study.

In isolated substrate-depleted hepatocytes the concentration of glycerol 3-phosphate may be critical for the rate of synthesis of triacylglycerol (Debeer *et al.*, 1981; Declercq *et al.*, 1982). Therefore the contents of glycerol 3-phosphate and glycogen were also measured.

Materials and methods

Hepatocytes were prepared from 16h-starved female Wistar rats (200g), essentially as described by Seglen (1973) and Mørland & Bessesen (1977). The liver was perfused (non-recirculating) *in situ* for 6 min with Krebs–Henseleit buffer (Krebs & Henseleit, 1932) from which CaCl₂ was omitted. The flow was gradually increased from 20 to 40 ml/min. Subsequently, the liver was perfused, with recirculation, for 10 min with Krebs–Henseleit buffer containing 5 mM-CaCl₂ and 0.05% (w/v) collagenase, and then non-recirculating for 2 min with original Krebs–Henseleit buffer. The liver was disrupted in 50 ml of Krebs–Henseleit buffer and

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transferred to a conical flask (1000 ml) in a shaking (90 cycles/min) water bath, and incubated for 10 min under O_2/CO_2 (19:1). The suspension was filtered through a 160 μ m-pore nylon filter and washed three times in a modified HI-WO/BA medium (Dashti *et al.*, 1980) by centrifugation at 50 *g* for 2 min. The hepatocytes excluded Trypan Blue by more than 85%.

Cells were plated [1.8×10^6 cells/50 mm Petri dish coated with rat tail collagen (Michalopoulos & Pitot, 1975)] in the modified HI-WO/BA medium supplemented with 10% (v/v) horse serum, insulin (0.1 μ M), dexamethasone (1 μ M), penicillin (100 units/ml) and streptomycin (0.1 mg/ml). The plated cells were incubated in air-tight chambers (22 cm $\times$ 22 cm $\times$ 26 cm) at 37°C with air/ CO_2 (19:1) as gas phase. At 4 h after plating the medium was changed, and the cells were incubated overnight (16 h) under the same conditions. On the next morning (day 2) the medium was replaced with serum-free medium and the medium was replaced every 24 h thereafter. Oleate complexed to albumin was added together with ethanol, insulin or dexamethasone as indicated. Total volume was 3 ml. All incubations were done in duplicate. Plates containing ethanol were placed in a separate air-tight chamber together with a beaker containing 500 ml of ethanol at an identical concentration.

In the morning at the time-points indicated (i.e. at the end of the 24 h periods), the medium was removed by suction and samples were taken for determination of non-esterified fatty acids (Ho, 1970) and of lactate (Hohorst, 1970). Krebs-Henseleit buffer (1.5 ml) was added and the cells were disrupted by sonication (Sonifier B-12; Branson, Danbury, CT, U.S.A.; 40 W for 10 s); samples were taken for determination of DNA (Kissane & Robins, 1958). Total acylglycerol was determined as glycerol after alkaline hydrolysis of a sample of the freeze-dried material (Kondrup *et al.*, 1980). For the separate determination of di- and tri-acylglycerol a sample of the freeze-dried material was extracted with chloroform/methanol (2:1, v/v) (Grunnet & Kondrup, 1983). Di- and tri-acylglycerol were quantified separately after t.l.c. (Kondrup, 1979). Separate cell cultures incubated in parallel were used for the determination of glycogen and glycerol 3-phosphate; these cultures were rinsed with warm Krebs-Henseleit buffer and 1.5 ml of 1.5% $HClO_4$ was added. After sonication (see above) and centrifugation (10 000 *g* for 2 min), the supernatant was used for determination of glycogen after enzymic hydrolysis (Katz *et al.*, 1976) and of glycerol 3-phosphate by a fluorimetric method (Lowry & Passonneau, 1972).

Oxidation of ethanol was measured as the conversion of [$1-^{14}C$]ethanol into non-volatile products (Grunnet *et al.*, 1973) in closed tissue flasks.

Tyrosine aminotransferase was measured as described by Granner & Tomkins (1970). For electron-microscopic examination the cells were fixed *in situ* in the Petri dish for 60 min in a mixture of 0.8% glutaraldehyde and 0.7% OsO_4 in 0.1 M-cacodylate buffer, pH 7.2, at 0°C, rinsed briefly with cacodylate buffer and stained overnight in 0.5% uranyl acetate. After dehydration with increasing concentrations of ethanol, the cultures were infiltrated overnight in a 1:1 (v/v) mixture of ethanol and Epon-812 followed by two incubations in Epon, each lasting 60 min. Finally, the cells were embedded in fresh Epon and the preparation was allowed to polymerize for 4 days at 40°C. After detachment of the Epon sheet from the dish, it was cut into small pieces and ultrasectioned perpendicularly to the sheet.

Collagenase (type II) was from Worthington Biochemical Corp., Freehold, NJ, U.S.A. Horse serum was from Gibco, Glasgow, Scotland, U.K. Albumin was from Armour, Eastbourne, Sussex, U.K., and was delipidated as described by Chen (1967). Insulin was purchased from Novo, Copenhagen, Denmark, and dexamethasone from Merck, Sharp and Dohme, Haarlem, The Netherlands. Enzymes and coenzymes were from C. F. Boehringer, Mannheim, Germany.

The rates of accumulation of triacylglycerol were calculated from the slopes resulting from regression analysis by using the least-squares method. Statistical significance was calculated by the *t* test for paired data.

Results

Cell survival was measured by the content of DNA per plate. From day 2 to 6 the content of DNA per plate declined by 3% per day in the absence of ethanol (with or without hormones). This is similar to the results of a 24 h study (Durrington *et al.*, 1982); the survival on day 4 in the present study was about 1.5-fold higher than in a previous 4-day study (Barth *et al.*, 1977). In the presence of ethanol the decrease in DNA was 5% per day. On day 6 the content of DNA in the presence of ethanol (with or without hormones) was $22.1 \pm 1.8 \mu$ g per plate, compared with $25.1 \pm 1.6 \mu$ g per plate in the absence of ethanol (mean $\pm$ S.E.M., $n = 7$; $P < 0.01$). The final content of DNA on day 6 in control plates (without ethanol and hormones) was 25.3 ± 2.9 , and in the presence of dexamethasone or insulin it was 24.2 ± 3.2 or 25.9 ± 2.9 respectively (μ g of DNA per plate; mean $\pm$ S.E.M., $n = 7$). These results indicate that the cell survival was decreased by ethanol and independent of the presence of hormones. At any time-point only 1–2% of the cells took up Trypan Blue.

The content of glycogen was measured throughout the experimental period (Figs. 1a–1c). Freshly

Fig. 1. Time course of content of glycogen, triacylglycerol and glycerol 3-phosphate in cultured hepatocytes. Isolated hepatocytes were cultured in a modified HI-WO/BA medium as described in the text in the absence (●) or in the presence (○) of 50 mM-ethanol. Oleate (1 mM) and albumin (0.29 mM) were added to all plates. Insulin (0.1 μ M) and dexamethasone (1 μ M) were added as indicated. Bars indicate S.E.M. (a–c), Glycogen (μ mol of glucosyl units/mg of DNA), $n = 3$. (d–f), Triacylglycerol (μ mol of glycerol/mg of DNA), $n = 7$. (g–i), Glycerol 3-phosphate (μ mol/mg of DNA), $n = 3$.

isolated cells contained about 0.3 μ mol of glucosyl units/mg of DNA. After the first 24 h the content had risen to about 80 μ mol of glucosyl units/mg of DNA (day 2, Figs. 1a–1c). By assuming a DNA content of 2.3 mg/g wet wt. (Fischer-Szafarz *et al.*, 1981), this content can be recalculated to about 180 μ mol of glucosyl units/g wet wt. In liver from fed rats we previously found 136 μ mol of glucosyl units/g wet wt. (Kondrup *et al.*, 1980). At later time-points, depending on the presence of hormones, the content of glycogen declined; the final content was still about 10 times that in the freshly isolated cells. With ethanol present the content of glycogen declined from day 2, irrespective of the presence of hormones; *in vivo* it is well known that prolonged ethanol feeding decreases the hepatic content of glycogen (see, e.g., Kondrup *et al.*, 1980).

Ethanol was oxidized at rates between 0.61 (day 2) and 0.66 (day 4) μ mol/min per mg of DNA (results not shown). The rates were similar at 20 mM-

and 80 mM-ethanol. These rates are equivalent to about 1.5 μ mol/min per g wet wt., which is about 75% of the rate measured in freshly isolated hepatocytes from fed rats (Selmer & Grunnet, 1976).

Insulin increased the glycogen content throughout the experimental period. On days 4, 5 and 6 the content was statistically significantly higher in the absence of ethanol ($P < 0.001$); in the presence of ethanol the content of glycogen was not consistently increased by insulin (Figs. 1a and 1b). Addition of dexamethasone increased the activity of tyrosine aminotransferase from 0.21 to 0.78 unit/mg of DNA ($n = 2$, measured on day 3; results not shown).

Ultrastructurally the cultures exhibited bile canaliculi and a well-preserved structure after 24 h (Plate 1a). On day 6 the cells contained numerous small vesicles and a few autophagic vacuoles, and the endoplasmic reticulum was dilated (Plate 1b). Similar observations have been published by others (Chapman *et al.*, 1973; Wanson *et al.*, 1977).

Ethanol had no obvious effect on the organelle structure.

Accumulation of triacylglycerol

The accumulation of total acylglycerol was linear with time, as shown in Figs. 1(d)–1(f). Triacylglycerol was responsible for the increase in acylglycerol, whereas diacylglycerol remained constant; this was determined in three experiments on days 2, 3 and 5 (results not shown). The sum of di- and tri-acylglycerol accounted for 85–90% of total acylglycerol (see the Materials and methods section). These observations applied to all experimental conditions, and therefore in the following text 'triacylglycerol' is used synonymously with 'total acylglycerol'.

In Table 1 are shown the rates of accumulation of triacylglycerol as calculated from the daily increments in triacylglycerol (slopes in Figs. 1d–1f). The accumulation proceeded linearly in all experiments, the lowest *r* value being 0.97. Addition of ethanol increased the rate of accumulation by 90%; insulin and dexamethasone increased the rate by 50% and 30% respectively. With insulin or dexamethasone present, ethanol increased the rate by only about 40%, yielding rates that were similar to that after addition of ethanol alone. When insulin and dexamethasone were added simultaneously, the rate of accumulation was similar to that after addition of insulin alone; further addition of ethanol increased the rate to about the same value as that with ethanol alone (results not shown). The effect of ethanol was maximal already at 10 mM-ethanol, the lowest concentration tested.

Within the 24 h period the concentration of fatty acids declined exponentially, and the final concentration was about 0.2 mM (results not shown). The uptake of fatty acids was about 80 μ mol/24 h per mg of DNA; the maximal rate of accumulation of triacylglycerol was thus about 45% of the fatty acid uptake.

Table 1. *Accumulation of triacylglycerol in cultured hepatocytes*

The rates of accumulation were calculated by linear regression analysis of the results from days 2 to 6 (see Figs. 1d–1f). The results are means $\pm$ S.E.M., *n* = 7.

	Rate of accumulation (μ mol/day per mg of DNA)		
	No hormone	+ Insulin	+ Dexamethasone
Control	6.2 $\pm$ 0.8	9.2 $\pm$ 1.3†	8.0 $\pm$ 0.8‡
+ Ethanol	11.8 $\pm$ 1.2**	12.3 $\pm$ 1.2**	12.3 $\pm$ 1.5*

* *P* < 0.01 compared with control without ethanol.

** *P* < 0.001 compared with control without ethanol.

† *P* < 0.02 compared with control without hormone.

‡ *P* < 0.01 compared with control without hormone.

On day 6 the content of triacylglycerol in the presence of ethanol was equivalent to about 120 μ mol/g wet wt. In a particularly lipid-rich subpopulation of hepatocytes isolated from rats given ethanol for 4 weeks, the content of triacylglycerol was equivalent to about 140 μ mol/g wet wt. (Kondrup *et al.*, 1980).

The accumulation of triacylglycerol, in the presence or absence of ethanol, was accompanied by the appearance of cytoplasmic lipid droplets (Plate 1).

Glycerol 3-phosphate

The variations in the cellular concentration of glycerol 3-phosphate are shown in Figs. 1(g)–1(i). Without ethanol there was a slight increase from day 2 to day 3, and then the concentration declined to about 0.2 μ mol/mg of DNA, corresponding to about 0.4 μ mol/g wet wt. In liver from 8 h-starved rats we previously found a concentration of glycerol 3-phosphate of about 0.2 μ mol/g wet wt. (Kondrup & Grunnet, 1973).

The hormones had only small effects on the concentration of glycerol 3-phosphate. In the presence of ethanol the concentration of glycerol 3-phosphate was increased about 8-fold from day 2 to day 3 and then decreased to about 0.3 μ mol/mg of DNA.

Discussion

Evaluation of the cell culture

The cells maintained metabolic integrity throughout the experimental period, as suggested by the DNA content, the lack of Trypan Blue uptake, the constant rate of ethanol oxidation and the linear accumulation of triacylglycerol. Also, the effect of insulin on the glycogen content and the effect of insulin and dexamethasone on the accumulation of triacylglycerol were demonstrable throughout the experimental period. Dexamethasone increased the activity of tyrosine aminotransferase. The ultrastructure, in contrast, showed some degenerative alterations and the cultures were unable to sustain the initial high contents of glycogen and glycerol 3-phosphate.

Accumulation of triacylglycerol

The control hepatocytes accumulated triacylglycerol, which is in contrast with the situation with normal rats *in vivo*. The average fatty acid uptake during the 24 h interval can be recalculated to 0.13 μ mol/min per g wet wt., compared with about 0.2 μ mol/min per g wet wt. measured *in vivo* (Abrams & Cooper, 1976a); however, during the first 2 h of the 24 h interval the rate of fatty acid uptake was about 4-fold higher and coincided with the maximal rate of accumulation of triacylglycerol (results not shown). Therefore a high initial rate of

EXPLANATION OF PLATE 1

Ultrastructure of cultured hepatocytes

Cultured hepatocytes were sectioned perpendicularly to the bottom of the Petri dish. (a) Hepatocytes from day 2. Bile canaliculi (bc) were formed. Both smooth (ser) and rough (rer) endoplasmic reticulum was present. Also shown: cytoplasmic lipid droplets (ld), peroxisome (p), mitochondria (m). (b) Hepatocytes from day 6. The cytoplasm exhibited numerous small vesicles (v) and free ribosomes (r). Bar indicates 1 μ m.

synthesis combined with a comparatively slow turnover rate may explain this finding. The increase in the rate of accumulation of triacylglycerol caused by ethanol was similar to the rate of accumulation *in vivo* when a fatty liver is rapidly developing; single large doses of ethanol *in vivo* caused triacylglycerol to accumulate at rates that can be recalculated to 5–10 $\mu\text{mol}/24\text{ h}$ per mg of DNA (Brodie *et al.*, 1961; Maling *et al.*, 1967).

Accumulation of triacylglycerol is determined by the balance between synthesis and degradation. Synthesis is determined by availability of substrates and by enzyme activity. Concentrations of glycerol 3-phosphate below 0.4 $\mu\text{mol}/\text{g}$ wet wt. were found to limit synthesis of triacylglycerol in substrate-depleted hepatocytes (Debeer *et al.*, 1981; Declercq *et al.*, 1982). However, the critical concentration of glycerol 3-phosphate may depend on the tissue preparation; in perfused liver from starved rats, inhibition of β -oxidation restored the rate of esterification to values for fed animals at a glycerol 3-phosphate concentration as low as 0.13 $\mu\text{mol}/\text{g}$ wet wt. (McGarry & Foster, 1971; McGarry *et al.*, 1973). This was not possible in isolated hepatocytes from starved rats at a concentration of glycerol 3-phosphate of approx. 0.16 $\mu\text{mol}/\text{g}$ wet wt. (Debeer *et al.*, 1981).

In the present study, the lowest concentration of glycerol 3-phosphate was measured on day 6 in the presence of dexamethasone (Fig. 1*i*). The concentration was equivalent to about 0.2 $\mu\text{mol}/\text{g}$ wet wt.; still, the rate of accumulation of triacylglycerol was continuously higher than that of control cells, which exhibited a higher concentration of glycerol 3-phosphate on day 6. This suggests that the concentration of glycerol 3-phosphate was not critical under the present conditions.

The availability of fatty acids is a major determinant of the rate of accumulation of triacylglycerol (Heimberg *et al.*, 1969). Accordingly we found that the rate increased severalfold when the concentration of oleate was increased within the range 0.2–1.2 mM (results not shown).

The effect of insulin in the present study may be explained by increased availability of fatty acids for esterification and storage. Insulin is known to increase fatty acid synthesis, even in the presence of 1 mM-oleate (Topping & Mayes, 1982); insulin also decreases fatty acid oxidation (Topping & Mayes, 1972), and it decreases ketogenesis in cultured hepatocytes (Harano *et al.*, 1982). In cultured hepatocytes, insulin increases the cellular content of triacylglycerol concomitantly with a decreased secretion of triacylglycerol (Durrington *et al.*, 1982). All these effects of insulin would increase the availability of triacylglycerol for storage.

Increased activity of phosphatidate phosphohydrolase may be responsible for the increased

accumulation of triacylglycerol in the presence of dexamethasone (Fallon *et al.*, 1977; Brindley *et al.*, 1977; Jennings *et al.*, 1981). Phosphatidate phosphohydrolase was not measured in the present study, but the activity of tyrosine aminotransferase was increased 4-fold in the presence of dexamethasone; Jennings *et al.* (1981) found the two enzymes to increase in parallel after addition of dexamethasone. When insulin and dexamethasone were present simultaneously, the rate of triacylglycerol accumulation was similar to that after addition of insulin alone. This may reflect the antagonistic effects of insulin and glucocorticoids on the activity of phosphatidate phosphohydrolase (Lawson *et al.*, 1982). Thus the effects of the hormones on the accumulation of triacylglycerol are explicable by their known effects on the synthetic pathway, but in addition possible effects on the degradation should be considered.

Effect of ethanol

It is not clear from the present investigation how ethanol increased the accumulation of triacylglycerol. Ethanol did not increase the uptake of fatty acids ($n = 4$, results not shown). Esterification could be increased relative to oxidation by the increased content of glycerol 3-phosphate or by inhibition of β -oxidation (see Baraona & Lieber, 1979). A possible increase in lactate could also decrease β -oxidation (McGarry & Foster, 1971). However, the effect of ethanol can hardly be explained by changes in any of these parameters. Thus, with time glycerol 3-phosphate decreased almost to control values (Fig. 1*g*), whereas the ethanol-induced increase in triacylglycerol accumulation continued to be as marked as at earlier time-points. Inhibition of β -oxidation by ethanol was associated with an unaltered accumulation of triacylglycerol in perfused rat liver (Kondrup *et al.*, 1979*a,b*) and with a modest 9% increase in the fractional esterification of exogenous palmitate in isolated hepatocytes (Grunnet & Kondrup, 1983), compared with the present 90% increase in triacylglycerol accumulation. Finally, on days 3 and 4, the mean lactate concentration in the culture medium was 6.2 and 3.5 mM in the absence and presence of ethanol respectively (results not shown).

The observations of the present study are consistent with some previous results obtained with cultured hepatocytes (Wood & Lamb, 1979). They found increased incorporation of [1,3- ^{14}C]glycerol into triacylglycerol 6 h after addition of ethanol. However, after 24 h, ethanol (at concentrations above 1 mM) decreased the incorporation into triacylglycerol; simultaneously, the activities of enzymes involved in glycerolipid synthesis had declined and ethanol oxidation apparently had ceased. The internal inconsistency of their results

may be due to a lower viability of the cells in their system.

It is unexplained why the rate of triacylglycerol accumulation was about the same in the presence of ethanol alone as in the presence of ethanol plus hormones. The hormones and ethanol may have antagonistic effects, the accumulation process may be saturated in the presence of ethanol alone or a step beyond the control of these compounds may become limiting. It is not likely that lack of fatty acids limited the effect of hormones, since, in the presence of ethanol and hormones, about 55% of the fatty acids taken up was metabolized by other routes than accumulation of triacylglycerol.

In vivo, a single large dose of ethanol causes fatty acids of adipose-tissue origin to accumulate as hepatic triacylglycerol (Lieber *et al.*, 1966). The main event is probably increased hepatic fatty acid uptake, which is partly due to a hormone-dependent increase in peripheral lipolysis (Maickel & Brodie, 1963) and partly due to increased hepatic blood flow (Abrams & Cooper, 1976a,b). Further, it has been suggested that the increase in glucocorticoids may affect the liver directly by increasing the hepatic activity of phosphatidate phosphohydrolase (Brindley *et al.*, 1979). However, in the present investigation dexamethasone failed to increase the effect of ethanol. In the present system the effect of ethanol was not associated with increased fatty acid uptake, and therefore the observed effect of ethanol may not be relevant for the acute fatty liver *in vivo*. During prolonged ethanol treatment, the diet, rather than adipose tissue, is the source of fatty acids accumulating as hepatic triacylglycerol (Lieber *et al.*, 1966; Mendenhall, 1972). The reason for this accumulation is unexplained (see, e.g., discussion by Kondrup *et al.*, 1979b); it remains to be elucidated to what extent the present system is an appropriate model for the situation *in vivo* during prolonged ethanol treatment.

For the comparison of the present system with the situation *in vivo* it is stressed that the cultured hepatocytes exhibited rates of accumulation of triacylglycerol that are comparable with those seen *in vivo* and that triacylglycerol accumulated to the same extent as *in vivo* after prolonged ethanol ingestion. Also, addition of insulin and dexamethasone increased the accumulation of triacylglycerol, which resembles the situation *in vivo*, since administration of cortisone to rats and hyperinsulinism in genetically obese mice are associated with fatty liver (Wiener *et al.*, 1968; Chan & Exton, 1977).

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References

- Abrams, M. A. & Cooper, C. (1976a) *Biochem. J.* **156**, 33-46
- Abrams, M. A. & Cooper, C. (1976b) *Biochem. J.* **156**, 47-54
- Baraona, E. & Lieber, C. S. (1979) *J. Lipid Res.* **20**, 289-315
- Barth, C., Willershausen, B., Walther, B. & Weis, E. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358**, 211
- Brindley, D. N., Bowley, M., Sturton, R. G., Pritchard, P. H., Burditt, S. L. & Cooling, J. (1977) *Biochem. Soc. Trans.* **5**, 40-43
- Brindley, D. N., Cooling, J., Burditt, S. L., Pritchard, P. H., Pawson, S. & Sturton, R. G. (1979) *Biochem. J.* **180**, 195-199
- Brodie, B. B., Butler, W. M., Horning, M. G., Maickel, R. P. & Maling, H. M. (1961) *Am. J. Clin. Nutr.* **9**, 432-435
- Chan, T. M. & Exton, J. H. (1977) *Biochim. Biophys. Acta* **489**, 1-14
- Chapman, S. C., Jones, A. L., Meyer, V. A. & Bissell, M. (1973) *J. Cell Biol.* **59**, 735-747
- Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173-181
- Dashti, N., McConathy, W. J. & Ontko, J. A. (1980) *Biochim. Biophys. Acta* **618**, 347-358
- Debeer, L. J., Declercq, P. E. & Mannaerts, G. P. (1981) *FEBS Lett.* **124**, 31-34
- Declercq, P. E., Debeer, L. J. & Mannaerts, G. P. (1982) *Biochem. J.* **204**, 247-256
- Durrington, P. N., Newton, R. S., Weinstein, D. B. & Steinberg, D. (1982) *J. Clin. Invest.* **70**, 63-73
- Fallon, H. J., Lamb, R. G. & Subhashchandra, C. J. (1977) *Biochem. Soc. Trans.* **5**, 37-40
- Fellenius, E., Bengtsson, G. & Kiessling, K.-H. (1973) *Acta Chem. Scand.* **27**, 2893-2901
- Fischer-Szafarz, B., Szafarz, D. & de Murillo, A. G. (1981) *Anal. Biochem.* **110**, 165-170
- Granner, D. K. & Tomkins, G. M. (1970) *Methods Enzymol.* **17A**, 633-637
- Grunnet, N. & Kondrup, J. (1983) *Pharmacol. Biochem. Behav.* **18**, Suppl. in the press
- Grunnet, N., Quistorff, B. & Thieden, H. I. D. (1973) *Eur. J. Biochem.* **40**, 275-282
- Harano, Y., Kosugi, K., Kashiwagi, A., Nakano, T., Hidaka, H. & Shigeta, Y. (1982) *J. Biochem. (Tokyo)* **91**, 1739-1748
- Heimberg, M., Weinstein, I. & Kohout, M. (1969) *J. Biol. Chem.* **244**, 5131-5139
- Ho, R. J. (1970) *Anal. Biochem.* **36**, 105-113
- Hohorst, H.-J. (1970) in *Methoden der Enzymatischen Analyse* (Bergmeyer, H.-U., ed.), pp. 1425-1429, Verlag Chemie, Weinheim
- Iseri, O. A., Lieber, C. S. & Gottlieb, L. S. (1966) *Am. J. Pathol.* **48**, 535-555
- Jennings, R. J., Lawson, N., Fears, R. & Brindley, D. N. (1981) *FEBS Lett.* **133**, 119-122
- Katz, J., Golden, S. & Wals, P. A. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3433-3437
- Kissane, J. M. & Robins, E. (1958) *J. Biol. Chem.* **233**, 184-188

- Kondrup, J. (1979) *Biochem. J.* **184**, 63–71
- Kondrup, J. & Grunnet, N. (1973) *Biochem. J.* **132**, 373–379
- Kondrup, J., Lundquist, F. & Damgaard, S. E. (1979a) *Biochem. J.* **184**, 83–88
- Kondrup, J., Lundquist, F. & Damgaard, S. E. (1979b) *Biochem. J.* **184**, 89–95
- Kondrup, J., Bro, B., Dich, J., Grunnet, N. & Thieden, H. I. D. (1980) *Lab. Invest.* **43**, 182–190
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Lawson, N., Jennings, R. J. & Brindley, D. N. (1982) *FEBS Lett.* **143**, 9–12
- Lieber, C. S., Spritz, N. & DeCarli, L. M. (1966) *J. Clin. Invest.* **45**, 51–62
- Lowry, O. H. & Passonneau, J. V. (1972) *A Flexible System of Enzymatic Analysis*, pp. 186–187, Academic Press, New York and London
- Maickel, R. P. & Brodie, B. B. (1963) *Ann. N.Y. Acad. Sci.* **104**, 1059–1064
- Maling, H. M., Highman, B., Hunter, J. M. & Butler, W. M. (1967) in *Biochemical Factors in Alcoholism* (Maickel, R. P., ed.), pp. 185–199, Pergamon Press, Oxford
- McGarry, J. D. & Foster, D. W. (1971) *J. Biol. Chem.* **246**, 6247–6253
- McGarry, J. D., Meier, J. M. & Foster, D. W. (1973) *J. Biol. Chem.* **248**, 270–278
- Mendelhall, C. L. (1972) *J. Lipid Res.* **13**, 177–183
- Michalopoulos, G. & Pitot, H. C. (1975) *Exp. Cell Res.* **94**, 70–78
- Mørland, J. & Bessesen, A. (1977) *Biochim. Biophys. Acta* **474**, 312–320
- Pritchard, P. H., Bowley, M., Burditt, S. L., Cooling, J., Glenny, H. P., Lawson, N., Sturton, R. G. & Brindley, D. N. (1977) *Biochem. J.* **166**, 639–642
- Pritchard, P. H., Cooling, J., Burditt, S. L. & Brindley, D. N. (1979) *J. Pharm. Pharmacol.* **31**, 406–407
- Seglen, P. O. (1973) *Exp. Cell Res.* **82**, 391–398
- Selmer, J. & Grunnet, N. (1976) *Biochim. Biophys. Acta* **428**, 123–137
- Topping, D. L. & Mayes, P. A. (1972) *Biochem. J.* **126**, 295–311
- Topping, D. L. & Mayes, P. A. (1982) *Biochem. J.* **204**, 433–439
- Wanson, J.-C., Drochmans, P., Mosselmans, R. & Ronveaux, M.-F. (1977) *J. Cell Biol.* **74**, 858–877
- Wiener, J., Loud, A. V., Kimberg, D. V. & Spiro, D. (1968) *J. Cell Biol.* **37**, 47–61
- Wirthenson, G., Brocks, D. G. & Guder, W. G. (1980) *Hoppe-Seyler's Z. Physiol. Chem.* **361**, 985–993
- Wood, C. K. & Lamb, R. G. (1979) *Biochim. Biophys. Acta* **572**, 121–131