

Effects of acetate, acetaldehyde, and ethanol on lipolysis in isolated rat adipocytes

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Abstract The effects of different concentrations of acetate, acetaldehyde, and ethanol, alone or in combination, on the lipolysis rate, measured as glycerol release, were studied in isolated adipocytes from fed or fasted rats, in the basal state and at various levels of norepinephrine stimulation. Acetate inhibited the glycerol release in a dose-dependent manner ($\approx 10\%$ inhibition at 2 mM, 25–70% at 10 mM) with the most marked effects at low to moderate norepinephrine concentrations (1.5–7 ng/ml). Little or no inhibition was observed in the absence of hormone and at maximal (100 ng/ml) hormone stimulation. Ethanol, up to 100 mM concentration, had no effect on the lipolysis rate. Acetaldehyde, up to 1 mM concentration, had no reproducible effect. Ethanol, acetaldehyde, and acetate in combination inhibited glycerol release to an extent similar to that of acetate alone.

Supplementary key words glycerol · spectrophotofluorometry · norepinephrine

The intake of small or moderate amounts of ethanol by man (1–4) or rats (5) is known to be rapidly followed by a decrease of plasma glycerol and free fatty acid concentrations. Since ethanol intake does not increase the uptake and utilization of glycerol (6, 7), this decrease must be the result of a lowered glycerol production, i.e., lipolysis rate in the adipose tissue.

The cause for the diminished lipolysis rate in adipose tissue has not been established. Intake of small or moderate amounts of ethanol has had no marked effects on the levels of lipolytic (8) or antilipolytic hormones (9). Infusion of acetate, the major metabolite of ethanol oxidation, into man decreased plasma FFA (10). It has therefore been suggested that acetate is responsible for the antilipolytic action of ethanol intake (11). However, *in vitro* experiments have given conflicting results. Both ethanol and acetaldehyde have been shown to have no influence or to have either stimulatory or inhibitory effects on lipolysis, depending on concentrations and other experimental conditions (12–14). Acetate slightly inhibited basal lipolysis in isolated adipocytes (15).

These divergent results prompted us to perform a

more systematic study of the effects of ethanol and its oxidation metabolites, acetaldehyde and acetate, on the lipolysis rate in adipose tissue. Glycerol release, in the basal state and at various stages of stimulation with the lipolytic hormone norepinephrine, has been used as a measure of this parameter. The isolated adipocytes from epididymal fat pads of fed or fasted rats were employed as a model system.

MATERIALS AND METHODS

Isolated fat cells were prepared by the collagenase digestion method of Rodbell (16) as modified by Gliemann (17) from epididymal fat pads and perirenal fat of 120–140 g male Sprague-Dawley rats (Anticimex, Stockholm, Sweden). The animals were either given laboratory chow (R3, Astra-Evos, Södertälje, Sweden) *ad libitum* or fasted overnight. The cells were suspended in Krebs-Ringer-HEPES buffer pH 7.4 (NaCl, 118.7 mM; KCl, 4.75 mM; CaCl₂, 2.54 mM; KH₂PO₄, 1.19 mM; MgSO₄, 1.19 mM; HEPES, 24.6 mM) containing 3.5% (w/v) bovine serum albumin and 2.5 mM glucose. Cell concentration was measured as packed cell volume (PCV) after centrifugation in microhematocrit tubes (18). One ml of PCV from fed rats corresponded approximately to 0.7 g of extracted lipids and 40 mg of protein. Hormone sensitivity of the cells was routinely checked by determining the insulin stimulation of [³-³H]glucose uptake and conversion into lipids (19). Only cells showing a 15–30-fold maximal insulin stimulation of this parameter were used (cf. ref. 19).

One ml portions (10–15 μ l PCV) of a pooled cell suspension were transferred to polyethylene counting vials for incubation. After preincubation for 15 min at 37°C, zero-time samples were taken and norepineph-

Abbreviations: FFA, free fatty acids; HEPES, 2-[4-(2-hydroxyethyl)-piperazinyl-(1)]-ethane-sulfonic acid; PCV, packed cell volume.

rine or other substances (20–50 μl , aqueous solution) were added. Incubations were performed in a metabolic shaker (80 cycles/min, stroke length 4 cm) at 37°C with air as gas phase for the desired time (60 min, unless otherwise stated). They were terminated by quickly separating fat cells from medium by floating them through a silicone oil layer (18) in a polypropylene centrifuge tube (1 min, 10,000 g , Beckman Microfuge B). After cutting the tube just below the oil layer, a portion of the medium was taken for glycerol analysis.

Glycerol analysis was performed with an enzymatic fluorometric method (20) modified to obtain higher sensitivity. Two hundred μl of incubation medium or glycerol standard solution (0–200 μM) was mixed with 200 μl each of ZnSO_4 (87 mM) and $\text{Ba}(\text{OH})_2$ (83 mM) and the precipitated proteins were removed by centrifugation. A 200 μl portion of the clear supernatant was then incubated for 75 min at room temperature with 300 μl of enzyme reagent mixture (8.7 ml of hydrazinium-HCl, 0.33 M, pH 9 with MgCl_2 , 1.5 mM; 1.0 ml of cystein, 35 mg/ml; 0.1 ml of EDTA, 0.1 M, pH 9; 0.2 ml of ATP, 20 mM; 0.2 ml of NAD, 30 mM; 1 μl

of glycerol kinase; and 10 μl of glycerol phosphate dehydrogenase) and 500 μl of 10 mM NaOH with 0.5 mM EDTA added. The fluorescence (λ_{exit} 340 and λ_{emit} 455 nm) was then determined. The glycerol standard curve was linear up to at least 100 μM and fluorescence was within 10% of expected NADH values. Glycerol release was calculated as the difference between the samples at the end of the incubation and the zero-time values and was expressed as $\mu\text{mol/ml}$ PCV per hr. The statistical comparison of control and experimental samples was made with Student's t test, comparison of means of two independent samples. Reproducibility within the same cell batch was 10% for basal and 2% for maximally stimulated lipolysis, and sensitivity ($2 \times \text{SD}$ of zero-time samples) was 0.3 nmol of glycerol, corresponding to 0.1 $\mu\text{mol/ml}$ PCV.

Norepinephrine bitartrate was from Apoteksbolaget, Stockholm, Sweden and acetaldehyde (99% pure) was from BDH Chemicals; bovine serum albumin (Cohn's fraction V) and NAD (grade V) were from Sigma Chem. Co, St. Louis, MO. Glycerokinase (E.C. 2.7.1.30), 1 mg/ml; glycerol-3-phosphate dehydrogenase (E.C.

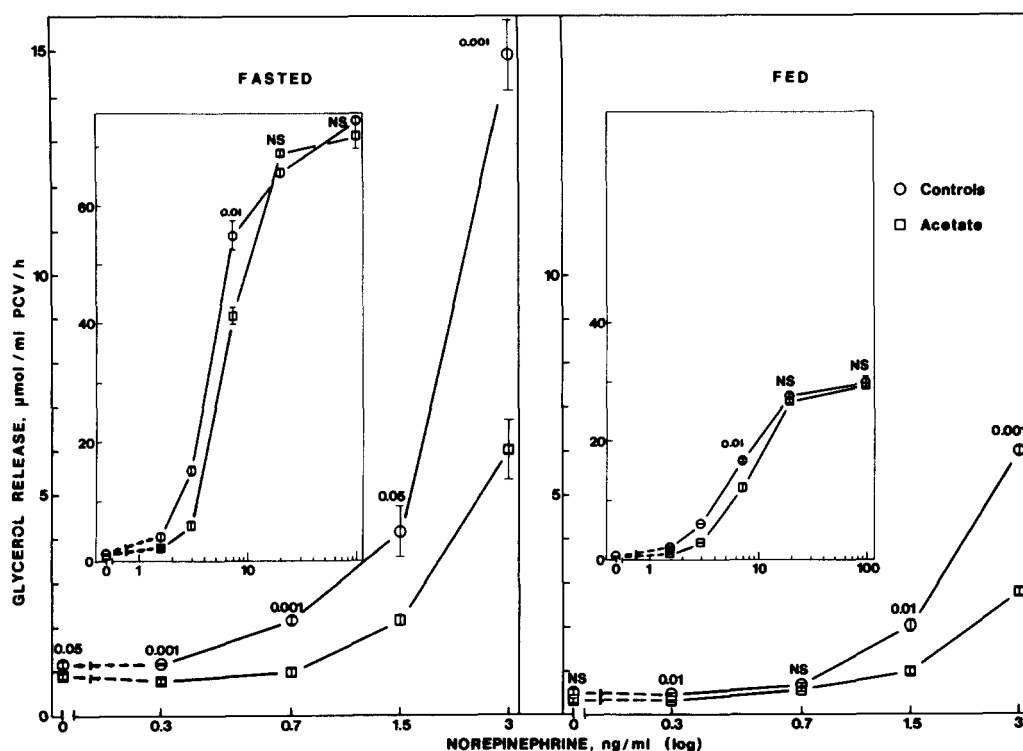


Fig. 1. Acetate inhibition of glycerol release at different norepinephrine concentrations. Adipocytes (12 μl PCV/ml) from fasted or fed rats were incubated (after 15 min preincubation) for 60 min with and without acetate (10 mM) at the indicated concentrations of norepinephrine in Krebs-Ringer-HEPES buffer, pH 7.4, with 3.5% albumin and 2.5 mM glucose at 37°C. The vials were shaken 80 cycles/min, stroke length 4 cm. Glycerol released to the medium was measured enzymatically—fluorometrically and related to the amount of adipocytes expressed as ml packed cell volume (PCV). Values are mean \pm SEM, $n = 3$ (number of samples from one cell batch), and the statistical significance was as indicated in the figure. N.S. signifies nonsignificant ($P > 0.05$).

1.1.1.8), 10 mg/ml; and ATP were from Biochimica Boehringer, Mannheim. Hydrazinium hydrochloride (24%) was from Merck, Darmstadt, and HEPES was from Schwarz-Mann.

The bovine serum albumin was extensively dialyzed against distilled water and filtered through a 0.8- μ m Millipore filter before use. The fatty acid content as determined by gas-liquid chromatography was approximately 0.25 mol per mol of albumin. Serum was prepared from rats fasted overnight.

RESULTS

Effects of acetate on glycerol release

Acetate (10 mM) inhibited glycerol release at various stages of norepinephrine stimulation with fat cells from both fed and fasted rats (Fig. 1). Little or no effect on basal or maximally hormone-stimulated glycerol release occurred, while inhibition was prominent (25–70%) at low to moderate norepinephrine concentrations. This pattern of inhibition by acetate was similar with cells from both fed and fasted rats, but there was a tendency for less effect with fat cells from fed rats at the lowest norepinephrine concentrations (Fig. 1). The acetate inhibition of moderately norepinephrine-stimulated glycerol release was constant with incubation time, possibly excepting the first few minutes (Fig. 2). It was clearly dependent on acetate concentration, roughly linear with the logarithm of this parameter, and with no difference due to nutritional state (Fig. 3). Statistically significant inhibition

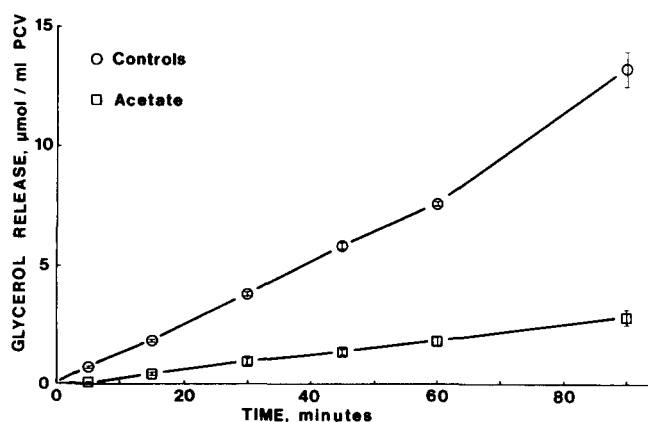


Fig. 2. Time course of acetate inhibition of norepinephrine-stimulated glycerol release. Adipocytes (15 μ l PCV/ml) from fed rats were incubated during the indicated time with and without acetate (20 mM) and with norepinephrine (3 ng/ml) under the conditions described in Fig. 1. Values are mean \pm SEM, $n = 3$. All values except those at the two shortest time intervals are statistically significant ($P < 0.01$).

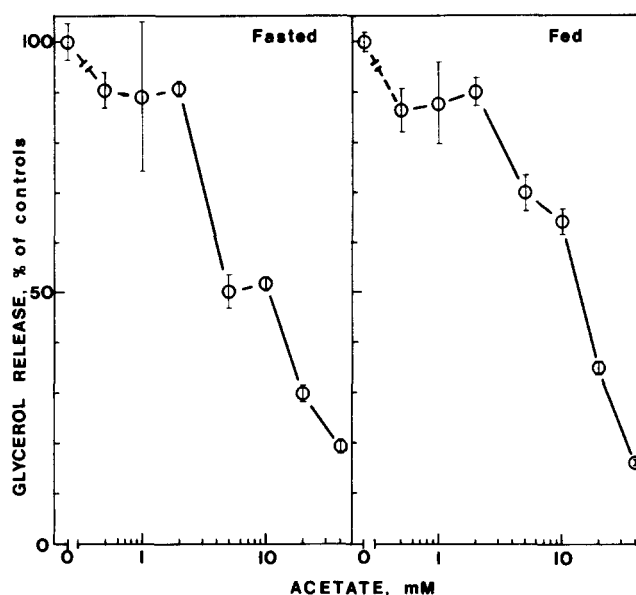


Fig. 3. Effects of varying acetate concentration on norepinephrine-stimulated glycerol release. Adipocytes from fasted and fed rats (15 μ l PCV/ml) were incubated for 60 min without and with the indicated concentrations of acetate and with norepinephrine (3 ng/ml) under the conditions described in Fig. 1. Values are mean \pm SEM, $n = 4$. Inhibition was statistically significant ($P < 0.05$) from 2 mM acetate concentration.

($P < 0.05$) could often be demonstrated at 1–2 mM acetate concentration.

Effects of ethanol

Ethanol (25 mM) had no statistically significant effect on glycerol release from fat cells from fed (Fig. 4) or fasted rats (data not presented), in the absence or presence of the indicated concentrations of norepinephrine. Furthermore, no effect could be observed with ethanol concentrations up to 100 mM at a moderately hormone-stimulated glycerol release rate (3 ng/ml of norepinephrine) (Table 1), while the high concentration of 400 mM (approx. 2% v/v) resulted in a three-fold increase of basal glycerol release but had little effect on the maximally hormone-stimulated rate. The reported ethanol inhibition of serum-stimulated glycerol release (14) was tested with 25 mM ethanol and serum diluted (1:1 v/v) with the incubation buffer. A slight (average 25%) but statistically significant ($P < 0.001$) decrease was obtained with cells from fasted rats (data not presented).

Effects of acetaldehyde and combined effects

Acetaldehyde (1–1000 μ M) had no reproducible effect on moderately (3 ng/ml) norepinephrine-stimulated glycerol release in any nutritional state. Acetaldehyde (100 μ M) had no reproducible effect on

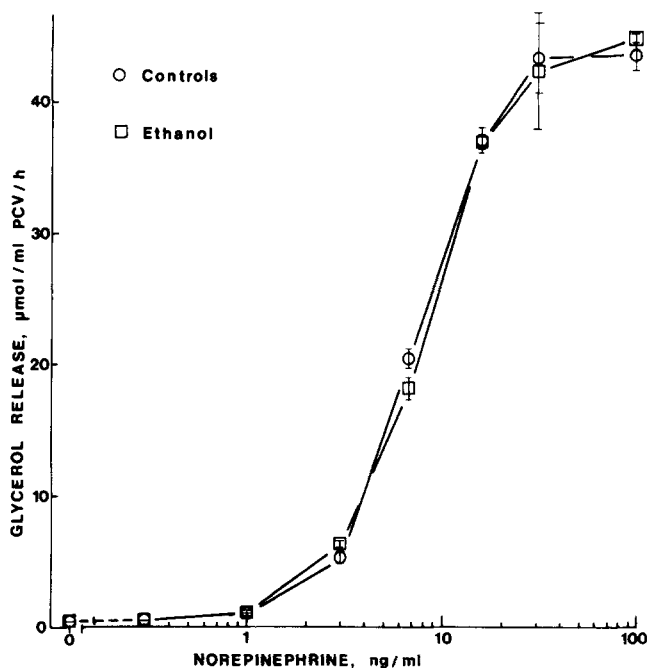


Fig. 4. Lack of effect of ethanol on glycerol release at different concentrations of norepinephrine. Adipocytes ($15 \mu\text{l}$ PCV/ml) from fed rats were incubated with and without ethanol (25 mM) at the indicated concentrations of norepinephrine under the conditions described in Fig. 1. Values are mean \pm SEM, $n = 3$. The difference between ethanol and control was in no case statistically significant ($P > 0.05$).

glycerol release at any stage of norepinephrine stimulation (1–100 ng/ml).

The inhibition of glycerol release by acetate (10 mM), ethanol (25 mM), and acetaldehyde (100 μM) in combination was similar to that of acetate (10 mM) alone (Table 2).

DISCUSSION

The high sensitivity of the modified enzymatic, fluorometric method for glycerol determination made

TABLE 1. Lack of effect of ethanol on glycerol release from isolated fat cells

Ethanol	Glycerol Release
mM	$\mu\text{mol/ml PCV/hr}$
0	14.4 ± 0.54
0.01	13.3 ± 0.55
0.1	14.5 ± 0.34
1	15.3 ± 0.63
10	16.6 ± 1.1
25	15.4 ± 0.30
100	14.6 ± 0.74

Adipocytes ($9.7 \mu\text{l}$ PCV/ml) from fed rats were incubated without and with ethanol at the indicated concentrations in the presence of norepinephrine (3 ng/ml) for 60 min under the conditions described in Fig. 1. Values are mean \pm SEM, $n = 5$.

TABLE 2. Effects of ethanol, acetaldehyde, and acetate alone or in combination on glycerol release from isolated fat cells

Addition	Glycerol Release	<i>P</i>
	% control	
None	100	
Ethanol, 25 mM	103.1 ± 6.9	N.S.
Acetaldehyde, 0.1 mM	99.4 ± 4.3	N.S.
Acetate, 10 mM	56.9 ± 6.1	<0.001
Ethanol + acetaldehyde + acetate, concentrations as above	62.6 ± 5.2	<0.001

Adipocytes representing seven separate cell batches from fasted rats were incubated with the additions stated above at the indicated concentrations and in the presence of norepinephrine (3 ng/ml) for 60 min under the conditions described in Fig. 1. Values are mean \pm SEM of the values obtained in the seven different experiments, each based on 3–5 incubations.

it possible to use as little as 10–15 μl PCV of fat cells in each incubation. This was of considerable practical value when a large number of incubations had to be performed with the same cell batch, e.g., in the experiments with the different norepinephrine concentrations described in Fig. 1.

The most significant finding of the present work was the consistent inhibition of glycerol release from adipocytes by acetate. This inhibition was observed at acetate concentrations comparable with those obtained in the circulating blood after ethanol ingestion (21) and at low to moderate hormone stimulation of lipolysis. These facts and the observation by others of a decrease of plasma free fatty acid concentration after acetate infusion into man (10) in combination with the lack of effects of ethanol and acetaldehyde on the lipolysis rate in this work indeed suggest that acetate is responsible for the antilipolytic effects of ingestion of small or moderate amounts of ethanol (1–5). The stimulatory effects of ethanol at 400 mM concentration reported by others (12) and also found by us is of little relevance in this connection, in view of the much lower concentrations obtained after ethanol ingestion. The same is true for the stimulatory effects of acetaldehyde at 1 mM concentration described by others in view of arterial blood levels of 20–160 μM (22) reported after ethanol ingestion. Lactate, which is produced at a higher than normal rate during ethanol metabolism (23) and which could theoretically influence the lipolysis in adipose tissue, has recently been shown to have no effect on this parameter in isolated fat cells (24).

The present work does not provide any evidence for the mechanism of the acetate effect. Acetate is known to be metabolized by adipose tissue (15). Most (50%) of the acetate produced during ethanol oxidation is believed to be oxidized by skeletal muscle (25)

but it is possible that a considerable part of the remainder may be disposed of by the adipose tissue. If so, acetate may have several effects on the intermediary metabolism of this tissue, which could possibly effect the rate of lipolysis. Alternatively, acetate, like several short-chain carboxylic acids and β -hydroxybutyrate (24), might inhibit adenosine 3':5'-monophosphate (cyclic AMP) accumulation, e.g., by interference with adenyl cyclase. ■

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REFERENCES

1. Lieber, C. S., C. M. Leevy, S. W. Stein, W. S. George, G. R. Cherrick, W. H. Abelmann, and C. S. Davidson. 1962. Effect of ethanol on plasma free fatty acids. *J. Lab. Clin. Med.* **59**: 826-832.
2. Nestel, P. J., and E. Z. Hirsch. 1965. Mechanism of alcohol-induced hypertriglyceridemia. *J. Lab. Clin. Med.* **66**: 357-365.
3. Feinman, L., and C. S. Lieber. 1967. Effect of ethanol on plasma glycerol in man. *Amer. J. Clin. Nutr.* **20**: 400-403.
4. Wolfe, B. M., J. R. Havel, E. B. Marliss, J. P. Kane, J. Seymour, and S. P. Ahuja. 1976. Effects of a 3-day fast and of ethanol on splanchnic metabolism of FFA, amino acids, and carbohydrates in healthy young men. *J. Clin. Invest.* **57**: 329-340.
5. Johnson, O. 1974. Influence of the blood ethanol concentration on the acute ethanol-induced liver triglyceride accumulation in rats. *Scand. J. Clin. Lab. Invest.* **33**: 207-213.
6. Lundquist, F., N. Tygstrup, K. Winkler, and K. B. Jensen. 1965. Glycerol metabolism in the human liver: Inhibition by ethanol. *Science*. **150**: 616-617.
7. Juhlin-Dannfelt, A. 1976. Effect of ethanol on substrate turnover in man. Thesis. University of Stockholm, Sweden. 1-57.
8. Perman, E. S. 1961. Effect of ethanol and hydration on the urinary excretion of adrenaline and noradrenaline and on the blood sugar of rats. *Acta Physiol. Scand.* **51**: 68-74.
9. Kuhl, C., and O. Andersen. 1974. Glucose- and tolbutamide-mediated insulin response after preinfusion with ethanol. *Diabetes*. **23**: 821-826.
10. Crouse, J. R., C. D. Gerson, L. M. DeCarli, and C. S. Lieber. 1968. Role of acetate in the reduction of plasma free fatty acids produced by ethanol in man. *J. Lipid Res.* **9**: 509-512.
11. Lieber, C. S. 1974. Effects of ethanol upon lipid metabolism. *Lipids*. **9**: 103-116.
12. Bizzi, A., and L. A. Carlson. 1965. Stimulatory effect of ethanol on glycerol release from rat adipose tissue in vitro. *Life Sci.* **4**: 2123-2128.
13. Giudicelli, Y., R. Nordmann, and J. Nordmann. 1972. Metabolic effects of acetaldehyde on rat adipose tissue in vitro. *Biochim. Biophys. Acta.* **280**: 393-407.
14. Curtis-Prior, P. B. 1972. Ethanol inhibition of serum stimulated lipolysis in isolated fat cells of the rat. *Experientia*. **28**: 1430-1431.
15. Flatt, J. P., and E. G. Ball. 1966. Studies on the metabolism of adipose tissue XIX. An evaluation of the major pathways of glucose catabolism as influenced by acetate in the presence of insulin. *J. Biol. Chem.* **241**: 2862-2869.
16. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239**: 375-380.
17. Gliemann, J. 1967. Assay of insuline-like activity by the isolated fat cell method. I. Factors influencing the response to crystalline insuline. *Diabetologia*. **3**: 382-388.
18. Gliemann, J., K. Østerlind, J. Vinten, and S. Gammeltoft. 1972. A procedure for measurement of distribution spaces in isolated fat cells. *Biochim. Biophys. Acta.* **286**: 1-9.
19. Moody, A. J., M. A. Stan, M. Stan, and J. Gliemann. 1974. A simple free fat cell bioassay for insulin. *Horm. Metab. Res.* **6**: 12-16.
20. Laurell, S., and G. Tibbling. 1965. An enzymatic fluorometric micromethod for the determination of glycerol. *Clin. Chim. Acta.* **13**: 317-322.
21. Juhlin-Dannfelt, A. 1977. Ethanol effects on substrate utilization by the human brain. *Scand. J. Clin. Lab. Invest.* **37**: 443-449.
22. Kissin, B., and H. Begleiter. 1971. The Biology of Alcoholism. Volume 1: Biochemistry. Plenum Press, New York. 165.
23. Wallgren, H., and H. Barry. 1970. Actions of Alcohol. Vol. 1. Elsevier, Amsterdam. 77-153.
24. Fain, J. N., and R. E. Shepherd. 1976. Inhibition of adenosine 3':5'-monophosphate accumulation in white fat cells by short chain fatty acids, lactate, and β -hydroxybutyrate. *J. Lipid Res.* **17**: 377-385.
25. Karlsson, N., E. Fellenius, and K.-H. Kiessling. 1975. The metabolism of acetate in the perfused hind-quarter of the rat. *Acta Physiol. Scand.* **93**: 391-400.