# Limitation in Glucose Penetration from the Liver into Blood and other Metabolic Symptoms of Ethanol Withdrawal in Rats

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Studies were undertaken to determine whether the distribution of glycolytic intermediates between the blood and liver in rats would be changed upon ethanol consumption and after its withdrawal. More drastic impairment of energy metabolism appeared to occur after ethanol withdrawal than upon chronic ethanol ingestion. The major metabolic manifestations of withdrawal were severe hypoglycemia, hyperlactatemia and dramatic hypopyruvatemia. The liver/blood gradient of glucose attained a value of 4.2 after ethanol withdrawal, suggesting that glucose penetration from the liver into circulation became limited. Besides, glycogen was accumulated in the liver of withdrawn animals despite the severe hypoglycemia.

*Ethanol consumption* *Ethanol withdrawal* *Energy metabolism* *Glycogen* *Glucose gradient* *(Rat liver, Blood)*

## 1. INTRODUCTION

Carbohydrate metabolism is generally known to be impaired upon ethanol administration. Hypoglycemia is a symptom of alcoholic intoxication in man and animals [1–5]. The pathophysiology of the condition is still not clear, although most researchers seem to agree that alcoholic hypoglycemia is the result of a decrease in glucose production by the liver [2–9]. Biochemical events in liver associated with withdrawal of alcohol consumption have received little attention. It has been commonly suggested that the effect of ethanol withdrawal on metabolism is a reversal of the ethanol effect [10,11]. However, no improvement of glycemia in abstinent patients as well as in alcoholic patients admitted to a hospital seems to be observed until glucose administration. Hypoglycemia can also be a symptom of alcohol withdrawal.

It is of great importance for further understanding of the mechanisms involved in alcoholic hypoglycemia and withdrawal glycemia to ascertain how both hepatic and plasma concentrations of glucose and its metabolites may change in relation to chronic ethanol consumption and sequential withdrawal. Here, we report the hepatic and blood levels of glucose, lactate and pyruvate, hepatic glycogen and other metabolites in rats after 2.5 months ethanol consumption and 24 and 48 h after ethanol withdrawal. The results show that hepatic glycogen stores failed to decrease in withdrawn animals under severe hypoglycemia. The liver/blood gradient of glucose as calculated on the basis of hepatic cytosolic and plasma water reached a value of 4.2 after withdrawal of ethanol consumption, suggesting that the glucose efflux from the liver into circulation became limited.

## 2. MATERIALS AND METHODS

### 2.1. *Animals and experimental design*

Male Wistar rats weighing 100–110 g were used initially and divided into two dietary groups. Animals in group 1 received ad libitum a balanced diet of 172 kcal/day, and those in group 2, a low-calorie corn mixture (1 g millet, 1 g wheat, 3 g sunflower seeds, 14 g oats, 4 g bread) of



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77 kcal/day. Two dietary groups were representatives of liver damage severity as most workers have observed fatty liver development in malnourished animals but not in well-fed ones [12].

Control rats had free access to water, and others to 10% (w/w) ethanol as the sole drinking liquid. The light-dark regimen was the natural one throughout the experiment performed over the period from February to April.

After 2.5 months ethanol intake, control and ethanol-fed rats were decapitated at 10.00–10.30 h. In some animals, after 3 months alcohol consumption, water was substituted for ethanol for 24 or 48 h prior to decapitation.

### 2.2. *Methods*

Within 7–10 s following decapitation, trunk blood was taken and a portion of the liver freeze-clamped at liquid nitrogen temperature. 0.5 ml blood was extracted immediately with a 1.5 ml mixture of 1.5 N perchloric acid/40% ethanol for 10 min at 0°C. The frozen liver tissue was crushed to a fine powder in a mortar under liquid nitrogen, rapidly weighed in the extraction mixture and extracted essentially as described in [13]. Preparation procedures and assays for metabolites were as in [13,14].

Results were treated statistically using Student's *t*-test.

### 2.3. *Materials*

Enzymes, coenzymes and substrates were purchased from Boehringer (Mannheim), Serva (Heidelberg) and BDH (London). Other reagents were of analytical grade and recrystallised.

## 3. RESULTS AND DISCUSSION

### 3.1. *Ethanol consumption by rats*

Animals of group 1 which had free access to a balanced diet consumed on average 7.2 ml/day of 10% ethanol on day 10, and rats of group 2, 9.3 ml/day. On the basis of kg body mass, these accounted for 7.5 and 9.7 g/day of pure ethanol, respectively. After 2.5 months ethanol consumption, the amount of ethanol solution taken in was increased to 13.5 ml/day in animals of group 1 and 12.0 ml/day in group 2. As their body mass rose to 308 and 240 g, the amount of pure ethanol taken in per kg body mass decreased significantly, being 4.38 and 5.00 g/kg per day, respectively.

### 3.2. *Chronic action of ethanol on blood and hepatic metabolites*

Tables 1 and 2 show that chronic ethanol consumption by rats of both dietary groups results in moderate hypoglycemia. Ketone bodies (mainly as 3-hydroxybutyrate) in blood and liver and the hepatic glycerol 3-phosphate level are increased, and the hepatic pyruvate concentration is decreased sharply in ethanol-fed animals. The malate content of the liver tends to increase but the difference between control and experimental animals is insignificant. These parameters as well as the reduced free cytosolic $NAD^+/NADH$ ratio calculated from the reactants of lactate dehydrogenase [15] are indicators of ethanol-induced changes in the redox state of liver cytosol and mitochondria [16–22].

Hepatic lactate concentration failed to increase after chronic ethanol consumption by rats of both groups (tables 1 and 2), in disagreement with a number of published data (e.g. [4,7,9,19,21,22]) but in line with others [10,17,23]. The reason for such a disagreement is unclear, but could be associated with different ages of the animals and conditioning.

Thus, chronic ethanol consumption by rats in this study results in a decrease in hepatic pyruvate, total lactate and pyruvate content and $NAD^+/NADH$ ratio of liver. The same has been observed by Gordon [10] in fatty livers of rats and Matsumura and Thurman [24] in perfused fed-rat liver. Analogous metabolic changes occur in the blood (tables 1,2). The liver/blood gradient of lactate indicates that blood lactate is extracted by the liver in control animals, and the gradient decreases after ethanol consumption. Pyruvate is distributed uniformly between the liver and blood in controls, but after ethanol consumption, the liver/blood gradient of pyruvate rises, apparently indicating the extraction of blood pyruvate by the liver.

### 3.3. *Effect of ethanol withdrawal on blood and hepatic metabolites*

On days 1 and 2 after withdrawal of ethanol consumption by rats, hepatic lactate and pyruvate concentrations, $NAD^+/NADH$ ratio as well as the blood lactate concentration change, as expected, in

Table 1

Blood and hepatic metabolite levels in rats fed a balanced diet after chronic alcohol ingestion and withdrawal

| Metabolites | Animals | | | |
|---|---|---|---|---|
| | Control | Ethanol ingestion for 71–75 days | Ethanol ingestion for 89–91 days, then without ethanol for | |
| | | | 1 day | 2 days |
| In blood | | | | |
| Glucose | 5.60 ± 0.14 | 4.42 ± 0.44[a] | 4.10 ± 0.14[a] | 3.00 ± 0.19[a] |
| Lactate | 1.92 ± 0.25 | 1.38 ± 0.03[a] | 1.73 ± 0.18 | 2.83 ± 0.18[a] |
| Pyruvate | 71 ± 10 | 75 ± 11 | 3[a] | 29 ± 4[a] |
| Ketone bodies | 89 ± 2 | 142 ± 9[a] | 200 ± 11[a] | 197 ± 9[a] |
| In liver | | | | |
| Glycogen | 222 ± 12 | 153 ± 20[a] | 185 ± 16 | 165 ± 8[a] |
| Glucose | 8.55 ± 0.53 | 6.80 ± 0.27[a] | 5.60 ± 0.13[a] | 5.16 ± 0.33[a] |
| Glucose 6-phosphate | 302 ± 22 | 308 ± 100 | 140 ± 48[a] | 140 ± 27[a] |
| Lactate | 0.91 ± 0.04 | 0.83 ± 0.07 | 1.80 ± 0.32[a] | 2.08 ± 0.13[a] |
| Pyruvate | 83 ± 7 | 30 ± 4[a] | 75 ± 10 | 120 ± 5[a] |
| $NAD^+$/NADH ratio | 819 ± 27 | 303 ± 23[a] | 321 ± 24[a] | 521 ± 26[a] |
| Ketone bodies | 207 ± 28 | 291 ± 6[a] | 544 ± 34[a] | 573 ± 38[a] |
| Malate | 231 ± 62 | 334 ± 78 | 201 ± 39 | 290 ± 79 |
| Glycerol 3-phosphate | 139 ± 21 | 309 ± 41[a] | 321 ± 27[a] | 440 ± 29[a] |
| ATP | 1.79 ± 0.13 | 1.52 ± 0.14[b] | 1.56 ± 0.06[b] | 1.68 ± 0.04 |
| Energy charge | 0.792 ± 0.015 | 0.724 ± 0.028 | 0.778 ± 0.009 | 0.820 ± 0.006[b] |

[a] $P < 0.01$

[b] $P < 0.05$ vs control

Results given are means ± SD for 6 animals in each subgroup. Blood glucose and lactate, and hepatic glycogen, glucose, ATP and lactate are expressed as μmol/g, and other metabolites as nmol/g

the reverse direction to their changes upon ethanol action (tables 1,2). In contrast, the concentrations of blood glucose and pyruvate continue to fall to rather low levels, and blood and hepatic ketone body concentrations and hepatic glycerol 3-phosphate continue to rise. Moreover, hepatic lactate and pyruvate levels in rats of both groups and blood lactate concentration in animals of group 1, 2 days after ethanol withdrawal, are increased by a factor of 1.5–3 over the control values (tables 1,2). All the alterations in metabolite patterns are similar in animals of both groups, suggesting that such alterations are mainly due to the effects of ethanol consumption and withdrawal, rather than different dietary factors.

Pyruvate cannot be detected in the blood of both animal groups 24 h after ethanol withdrawal (tables 1,2), so that a high liver/blood gradient of pyruvate suggests that pyruvate penetration from the liver into blood is blocked, the same result being obtained 48 h after ethanol withdrawal. The liver/blood gradient of lactate is nearly 1.0 in animals of group 1 and represents lactate efflux from the liver into blood in group 2, in contrast to that in control and ethanol-fed rats.

Hence, ethanol withdrawal is not a simple reversal of the ethanol effect, but is also expressed in other disturbances in energy metabolism. In particular, ethanol withdrawal appears to potentiate ethanol-induced hypoglycemia and to result in hyperlactatemia and drastic hypopyruvatemia.

### 3.4. *Hepatic ATP and energy charge levels*

Lactate and pyruvate accumulation by liver of

Table 2

Blood and hepatic metabolite levels in rats fed a low-calorie diet after chronic alcohol ingestion and withdrawal

| Metabolites | Animals | | | |
|---|---|---|---|---|
| | Control | Ethanol ingestion for 71–75 days | Ethanol ingestion for 89–91 days, then without ethanol for | |
| | | | 1 day | 2 days |
| In blood | | | | |
| Glucose | 6.43 ± 0.33 | 5.23 ± 0.58[a] | 3.13 ± 0.15[a] | 2.60 ± 0.08[a] |
| Lactate | 1.17 ± 0.27 | 0.79 ± 0.05 | 1.25 ± 0.16 | 1.40 ± 0.17 |
| Pyruvate | 73 ± 14 | 29 ± 14[a] | 3[a] | 3[a] |
| In liver | | | | |
| Glycogen | 219 ± 11 | 213 ± 32 | 283 ± 28[a] | 295 ± 27[a] |
| Glucose | 6.56 ± 0.22 | 5.63 ± 0.54[b] | 6.13 ± 0.42 | 6.28 ± 0.41 |
| Glucose 6-phosphate | 223 ± 15 | 228 ± 29 | 253 ± 25 | 249 ± 50 |
| Lactate | 0.89 ± 0.03 | 0.67 ± 0.04[a] | 2.10 ± 0.17[a] | 2.76 ± 0.22[a] |
| Pyruvate | 62 ± 4 | 30 ± 6[a] | 140 ± 36[a] | 138 ± 9[a] |
| $NAD^+$/NADH ratio | 632 ± 14 | 406 ± 53[a] | 593 ± 60 | 450 ± 12[a] |
| Ketone bodies | 145 ± 7 | 263 ± 42[a] | 350 ± 19[a] | 373 ± 41[a] |
| Malate | 290 ± 39 | 331 ± 98 | 282 ± 33 | 614 ± 70[a] |
| Glycerol 3-phosphate | 93 ± 35 | 273 ± 99[a] | 307 ± 17[a] | 1243 ± 106[a] |
| ATP | 1.48 ± 0.29 | 1.35 ± 0.06 | 1.53 ± 0.04 | 1.66 ± 0.13 |
| Energy charge | 0.751 ± 0.012 | 0.730 ± 0.058 | 0.782 ± 0.012[b] | 0.759 ± 0.021 |

[a] $P < 0.01$

[b] $P < 0.05$ vs control

Results given are means for 6 animals in each subgroup. Blood glucose and lactate, and hepatic glycogen, glucose, ATP and lactate are expressed as μmol/g, and other metabolites as nmol/g

Table 3

Ratio of liver content (μmol/g wet mass) to blood content (μmol/g) of glucose, calculated concentrations of glucose in liver cytosol water, [A], and that in plasma water, [D], and [A]/[D] ratio in rats fed a balanced diet (group 1) or a low-calorie diet (group 2) after chronic alcohol ingestion and withdrawal

| Parameter | Animals | | | |
|---|---|---|---|---|
| | Control | Ethanol ingestion for 71–75 days | Ethanol ingestion for 89–91 days, then without ethanol for | |
| | | | 1 day | 2 days |
| Group 1 | | | | |
| Liver/blood ratio | 1.53 | 1.54 | 1.37 | 1.72 |
| [A] (mM) | 16.52 | 13.16 | 10.52 | 10.23 |
| [D] (mM) | 6.67 | 5.26 | 4.88 | 3.57 |
| [A]/[D] | 2.48 | 2.50 | 2.16 | 2.87 |
| Group 2 | | | | |
| Liver/blood ratio | 1.02 | 1.08 | 1.96 | 2.42 |
| [A] (mM) | 14.33 | 9.82 | 12.45 | 13.17 |
| [D] (mM) | 7.65 | 6.23 | 3.73 | 3.10 |
| [A]/[D] | 1.87 | 1.58 | 3.34 | 4.25 |

Results given are means for 6 animals in each subgroup

ethanol-withdrawn rats suggests that hepatic glycolysis is enhanced. If so, one would expect that the steady-state concentration of ATP and the energy charge of the hepatic adenylate pool should be increased. Table 3 lists these data for livers of both animal groups. Both ATP and energy charge levels are decreased in rats of group 1 and tend to decrease in animals of group 2 after ethanol consumption. This is in agreement with previous observations on chronic ethanol effects in rats [21,22], in perfused rat liver [23] (concerning the energy charge, results were not given by the authors and were recalculated by us), and in fatty liver [10]. The hepatic ATP concentration and energy charge are restored on day 1 and maintained on day 2 after ethanol withdrawal (tables 1,2). Thus, if liver glycolysis is activated after ethanol withdrawal, the extent of activation would be insufficient to provide abundant lactate and pyruvate accumulation. The $NAD^+$/NADH ratio is increased partially after ethanol withdrawal but on day 2 is much lower than in controls, as expected for enhanced gluconeogenesis [25].

### 3.5. *Hepatic glycogen, glucose and glucose 6-phosphate*

Excess lactate and pyruvate and the reduced $NAD^+$/NADH ratio in liver of rats of both groups after ethanol withdrawal are factors favourable to increased gluconeogenesis. However, these parameters change in the same direction between days 1 and 2 as they do during day 1, while the hepatic ATP concentration and adenylate energy charge alter slightly and blood glucose decreases between days 1 and 2. This implies that some rate-limiting stages in glucose production arose. To elucidate these stages we measured hepatic glycogen, glucose and glucose 6-phosphate in rats of all subgroups (tables 1,2). In animals of group 1, glycogen decreases by 30% ($P < 0.01$) upon ethanol consumption and increases by 9–16% (insignificantly) after ethanol withdrawal. In rats of group 2, liver glycogen was virtually unchanged in ethanol intoxication but was elevated by 30–35% ($P < 0.01$) after ethanol withdrawal. This means that hypoglycemia associated with ethanol withdrawal is not accompanied by glycogen breakdown. In other words, liver glycogen does not represent the source of blood glucose under severe hypoglycemia. The absence of glycogen breakdown additionally does not support increased liver glycolysis after ethanol withdrawal.

The behavior of liver glycogen and blood glucose appears to be similar to that in Von Gierke's disease, a genetic defect of liver glucose-6-phosphatase [26]. In our experiment, the glucose 6-phosphate content of liver was decreased by a factor of 2 after ethanol withdrawal in rats of group 1, but remained unchanged in group 2 (tables 1,2). This argues against the inhibition of glucose-6-phosphatase as, in such a case, glucose 6-phosphate would be expected to increase. The liver content of glucose changed over a narrow range under all conditions, decreasing by 15–20% ($P < 0.01$) in rats of group 1 after ethanol withdrawal (vs animals given ethanol continuously) and even increasing by 10–15% (insignificantly) in animals of group 2 (tables 1,2). The glucose/glucose 6-phosphate ratio remained at a nearly constant level throughout the experiment, being in the range 22–40 for group 1 and 24–29 for group 2, arguing against the inhibition of glucose-6-phosphatase. Such behavior of hepatic glycogen, glucose and glucose 6-phosphate may be explained as follows. In rats of group 2, liver gluconeogenesis seems to compete with glycogen synthesis, whereas in group 1, a fraction of glucose 6-phosphate synthesized can enter the pentose phosphate pathway to give rise to NADPH necessary for lipogenesis. Actually, fatty liver has been shown to develop in malnourished animals only [12], and the pyruvate/malate ratio, which reflects the free cytosolic $NADP^+$/NADPH ratio [27] and correlates with the lipogenic rate [25], was lower in the liver of rats of group 1 as compared with group 2.

### 3.6. *Calculation of the liver/blood gradient of glucose*

The distribution of glucose between the liver and blood seems to be of particular interest. It is generally accepted that glucose penetrates the hepatocyte membrane freely in both directions [28–30]. In our experiment, the ratio of liver content (in $\mu$mol/g wet mass) to blood content (in $\mu$mol/g) of glucose was 1.53 and 1.02 in intact rats of groups 1 and 2, respectively (table 3). These values are in reasonable agreement with the value of 1.0 proposed by Newsholme and Start [30] and suggested by Cahill et al. [28]. The liver/blood

ratio of glucose appeared to be unaffected by chronic ethanol consumption but was increased to 1.72 in rats of group 1 and 2.42 in animals of group 2, 2 days after ethanol withdrawal (table 3).

It is noticeable that the liver and blood differ in water content and in the compartmentation of glucose. The amount of water in rat liver has been reported to be about 70% [28,31,32] and that in plasma 94% [28,33]. In addition, intercellular water accounts for 34% of liver water [34], i.e. 0.238 ml/g, and intracellular and cytosolic water (the latter being 91% of the intracellular value [35]) amounted to 0.462 and 0.420 ml/g wet mass, respectively. Let us assume that the hematocrit is 40% and plasma, erythrocyte and hepatic intercellular concentrations of glucose (in mmol/l) are equal. Erythrocyte and plasma water are 0.663 ml [36] and 0.935 ml [28,33] per ml cells or plasma, respectively. Taking into account these values, equations for glucose concentration, [A], in the liver cytosolic water and glucose concentration, [D], in plasma water (in mmol/l) can be obtained:

$$[A] = (B - 0.29C)/0.42, \text{ and}$$

$$[D] = C/0.84,$$

where $B$ is the measured liver content of glucose (in $\mu$mol/g wet mass) and $C$, the measured blood glucose (in $\mu$mol/g blood).

The values of [A], [D] and [A]/[D] are given in table 3. The [A]/[D] ratio appeared to increase to 2.87 in rats of group 1 and 4.25 in animals of group 2, 2 days after withdrawal of ethanol consumption.

Thus, glucose penetration from the liver into blood was impaired severely after ethanol withdrawal, especially in rats consuming a low-calorie diet.

In conclusion, our results show that chronic ethanol consumption results in impairment of carbohydrate metabolism in rat liver, but withdrawal of ethanol consumption appears to exert a more disturbing effect on energy metabolism, this effect being not a simple reversal of the ethanol effects. The major symptoms of ethanol withdrawal are severe hypoglycemia, hyperlactatemia and the complete disappearance of blood pyruvate. A reason for the pathology could be a perturbation in the free permeability of the liver cell membrane to glucose, resulting in sharp limitation of glucose efflux from the liver into blood and in liver glycogen becoming no source of blood glucose under severe hypoglycemia. Gluconeogenesis seems to be mostly undisturbed but extraction of lactate, the gluconeogenic precursor, by the liver is probably decreased due to the decreased availability of glucose, the glycolytic substrate, to other tissues and cells. The absence of blood pyruvate when the liver accumulates abundant pyruvate after ethanol withdrawal suggests indirectly that pyruvate is also not consumed by the liver under normal physiological conditions.

## REFERENCES

[1] Clark, W.C., Wilson, J.E. and Hulpieu, H.R. (1961) Q. J. Stud. Alcohol 22, 365–373.
[2] Field, J.B., Williams, H.E. and Mortimore, G.E. (1963) J. Clin. Invest. 42, 497–506.
[3] Freinkel, N., Singer, D.L., Arky, R.A., Bleicher, S.J., Anderson, J.B. and Silbert, C.K. (1963) J. Clin. Invest. 42, 1112–1131.
[4] Freinkel, N., Arky, R.A., Singer, D.L., Cohen, A.K., Bleicher, S.J., Anderson, J.B., Silbert, C.K. and Foster, A.E. (1965) Diabetes 14, 350–361.
[5] Madison, L.L. (1968) Adv. Metab. Disord. 3, 85–109.
[6] Krebs, H.A. (1968) Adv. Enzyme Regul. 6, 467–480.
[7] Kreisberg, R.A., Siegal, A.M. and Owen, W.C. (1971) J. Clin. Invest. 50, 175–185.
[8] Lochner, C.S., Wulff, J. and Madison, L.L. (1967) Metabolism 16, 1–18.
[9] Searle, G.L., Shames, D., Cavalieri, R.R., Bagdade, J.D. and Porte, D. jr (1974) Metabolism 23, 1023–1035.
[10] Gordon, E.R. (1973) J. Biol. Chem. 248, 8271–8280.
[11] Kalant, H., Khanna, J.M. and Loth, J. (1970) Can. J. Physiol. Pharmacol. 48, 542–549.
[12] Lieber, C.S., Teschke, R., Hasumura, Y. and DeCarli, L.M. (1975) Fed. Proc. 34, 2060–2074.
[13] Kosenko, E.A. (1981) Biokhimiya 46, 1389–1395 (in Russian).
[14] Kaminsky, Yu.G., Kosenko, E.A. and Kondrashova, M.N. (1982) Comp. Biochem. Physiol. B73, 957–963.
[15] Williamson, D.H., Lund, P. and Krebs, H.A. (1967) Biochem. J. 103, 514–527.
[16] Rawat, A.K. (1968) Eur. J. Biochem. 6, 585–592.
[17] Zakim, D. (1968) Proc. Soc. Exp. Biol. Med. 129, 393–397.

[18] Forsander, O.A. (1970) Q. J. Stud. Alcohol 31, 550–570.
[19] Veech, R.L., Guynn, R. and Veloso, D. (1972) Biochem. J. 127, 387–397.
[20] Guynn, R.W. and Pieklik, J.R. (1975) J. Clin. Invest. 56, 1411–1419.
[21] Cascales, C., Santos-Ruiz, M.R., Cascales, M. and Santos-Ruiz, A. (1981) Ciênc. Biol. 6, 219–222.
[22] Cascales, M., Santos-Ruiz, M.R., Cascales, C. and Santos-Ruiz, A. (1982) Ann. Pharm. Fr. 40, 499–510.
[23] Williamson, J.R., Scholz, R., Browning, E.T., Thurman, R.G. and Fukami, M.H. (1969) J. Biol. Chem. 244, 5044–5054.
[24] Matsumura, T. and Thurman, R.G. (1984) Eur. J. Biochem. 140, 229–234.
[25] Gumaa, K.A., McLean, P. and Greenbaum, A.L. (1971) Essays Biochem. 7, 39–86.
[26] Walvoort, H.C. (1983) J. Inher. Metab. Dis. 6, 3–16.
[27] Veech, R.L., Eggleston, L.V. and Krebs, H.A. (1969) Biochem. J. 115, 609–619.
[28] Cahill, G.F. jr, Ashmore, J., Earle, A.S. and Zottu, S. (1958) Am. J. Physiol. 102, 491–496.
[29] Newsholme, E.A. (1976) Prog. Liver Dis. 5, 125–135.
[30] Newsholme, E.A. and Start, C. (1973) Regulation in Metabolism, John Wiley, London.
[31] Lee, H. and Hosein, E.A. (1981) Life Sci. 29, 135–141.
[32] Hjelm, M. and Arturson, G. (1982) Uppsala J. Med. Sci. 87, 99–109.
[33] Ruderman, N.B., Schmahl, F.W. and Goodman, M.N. (1977) Am. J. Physiol. 233, E109–E114.
[34] Iles, R.A., Baron, P.G. and Cohen, R.D. (1979) Biochem. J. 184, 635–642.
[35] Siess, E.A., Brocks, D.G. and Wieland, O.H. (1982) in: Metabolic Compartmentation (Sies, H. ed.) pp.235–257, Academic Press, New York.
[36] Benos, D.J. and Tosteson, D.C. (1980) Biochim. Biophys. Acta 601, 167–179.