Biochemical Pharmacology, Vol. 37, No. 11, pp. 2209–2213, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

HEPATIC CARBOHYDRATE METABOLISM IN RATS BRED FOR ALCOHOL PREFERENCE

O. A. FORSANDER* and A. R. PÖSÖ†‡

* Research Laboratories of the Finnish State Alcohol Company (Alko Ltd.), POB 350, SF-00101 Helsinki, Finland, and † Department of Biochemistry, College of Veterinary Medicine, POB 6, SF-00551 Helsinki, Finland

(*Received* 27 *July* 1987; *accepted* 7 *December* 1987)

Abstract—The influence of ethanol on the carbohydrate metabolism was studied in two strains of rats: the AA strain with an inherited preference for alcohol and the ANA strain with an aversion to alcohol. In both strains, a single intraperitoneal dose of ethanol (1.5 g/kg body wt.) slightly increased the blood glucose concentration. In AA rats alcohol increased the rate of gluconeogenesis from alanine and had no effect on the liver glycogen stores, whereas in ANA rats the rate of gluconeogenesis remained unchanged and the glycogen stores decreased. It thus appears that the two rat strains maintain their blood glucose concentration by different mechanisms; the ANA rats utilise both glycogenolysis and gluconeogenesis but the AA rats only gluconeogenesis.

The genetic linkage of voluntary alcohol consumption is well documented [1, 2], but the nature of the mechanism that regulates alcohol drinking is still unknown. Several investigators have suggested that a prediabetic state or a high blood glucose level is a prerequisite for high voluntary alcohol consumption in experimental animals [3–5]. Also in humans an association between diabetes and alcoholism has been demonstrated [6]. Contradictorily, some results show that not all prediabetic animals prefer alcohol [7] and that nondiabetic animals can consume large amounts of alcohol [8, 9].

The assumption that carbohydrate metabolism interacts with the regulation of alcohol-drinking behaviour is supported by metabolic studies. During alcohol oxidation the blood glucose level, the glycogen content of the liver and the rate of gluconeogenesis undergo changes the direction of which depends on the nutritional and physiological state of the animal [10–13]. These ethanol-induced changes affect the availability of glucose in the entire organism and carry fundamental importance for the central nervous system, erythrocytes and adrenal medulla, which rely mainly on carbohydrate metabolism for their energy production.

In this study the association of alcohol-drinking behaviour and carbohydrate metabolism was investigated by comparing the acute effect of ethanol on blood glucose concentration, on the rate of gluconeogenesis from alanine and on hepatic glycogen content in two rat strains, Alko Alcohol (AA) and Alko NonAlcohol (ANA), with a widely different inherited preference for alcohol. Part of this study has been published previously as an abstract [14].

MATERIALS AND METHODS

Animals. The AA and ANA rats used in this study have been outbred in the Alko laboratories (Helsinki, Finland) with respect to voluntary alcohol drinking. In a free choice situation the AA rats show high preference for ethanol whereas the ANA rats avoid it. The animals used in these experiments were female rats aged 3–4 months from the F46 and F47 generations [1].

All rats were housed in stainless steel cages, 5 or 6 in each cage, and fed laboratory rat food (R3, Ewos, Södertälje, Sweden) and tap water *ad libitum* until the experiments. It has been shown previously that there are no interstrain differences in the food consumption of the AA and the ANA rats when ethanol is not included in the diet [9]. The animal rooms were artificially illuminated from 6 a.m. to 6 p.m., the temperature was 22 ± 2° and relative humidity 55 ± 5%. All the experiments were started at 9 a.m. To minimise stress from handling, the rats were kept in the animal rooms until the start of the experiments.

Blood glucose and ethanol oxidation. The rate of ethanol oxidation and the effect of ethanol on blood glucose concentration were studied after intraperitoneal injection of 10% (w/v) ethanol in saline (1.5 g ethanol/kg body weight). Blood samples were taken from the tip of the tail 60, 120 and 150 min after the injection. Control rats received a corresponding volume of saline and the blood samples for glucose analysis were taken as in the ethanol-receiving group. The blood was mixed with ice-cold perchloric acid (0.6 M), centrifuged and the concentrations of glucose and ethanol in the supernatant were determined. Ethanol was measured by gas chromatography [15] and glucose by the glucose oxidase method [16] using a test kit from Boehringer Mannheim (FRG).

Gluconeogenesis. Gluconeogenesis from alanine was measured 150 min after an intraperitoneal injection of ethanol (1.5 g/kg body wt) or saline essentially as described by Ayuso-Parrilla *et al.* [17]. In

‡ To whom correspondence should be addressed.

brief the method is as follows. The rats were anaesthetised with pentobarbital (60 mg/kg to the saline controls and 40 mg/kg to the ethanol rats) 140 min after the ethanol/saline injection. When the anaesthetic had taken effect, their abdomen was opened and tracer amounts of ^{14}C-alanine (U-^{14}C-L-alanine, specific activity 160 mCi/mg) in saline (2 μCi/rat) was injected into the portal vein. Exactly 5 min later a blood sample was drawn from the aortic bifurcation into a heparinised syringe for the determination of alanine and glucose concentrations in blood and the ^{14}C content of the blood alanine and glucose.

The aortic blood was centrifuged and part of the plasma was precipitated with perchloric acid and used for the determination of alanine and glucose. The radioactivities of alanine and glucose were determined from the remaining plasma as follows. Alanine was first extracted from the plasma with Dowex 50W ion exchange resin [18, 19]. The resulting amino-acid-free plasma was further treated with Dowex 2 resin. Radioactivity in glucose, measured from the resin-treated (Dowex W50 and Dowex 2) plasma, was corrected for the dilution of the sample as in Mallette *et al.* [18]. Alanine was eluted from the Dowex 50W resin with 5 M NH_4OH [19]. The ammonia was removed by evaporating the solution to dryness in a boiling water bath. The amino acids were dissolved in distilled water from which the radioactivity in alanine was determined.

Concentration of alanine was measured with alanine dehydrogenase [20] and that of glucose as above [16]. Because hepatic uptake of alanine is linearly related to the concentration in portal vein [21], the specific radioactivity of alanine in plasma was assumed to reflect the specific radioactivity in liver and, together with the radioactivity incorporated into glucose, was used to calculate the rate of gluconeogenesis that is expressed as nmoles alanine incorporated per 1 ml plasma in 5 min. For calculation of the relative contribution of glycogenolysis and gluconeogenesis to glucose production, it was assumed that the newly synthesised glucose equilibrates rapidly and evenly in the extracellular water space that was assumed to be 30% [22].

Hepatic glycogen and alanine. After the blood sample for the above measurements was drawn, the liver was quickly removed and frozen in liquid nitrogen for glycogen determination. Glycogen was determined according to Van Handel [23].

Concentration of hepatic alanine was measured in freeze-clamped liver samples 150 min after ethanol (1.5 g/kg body wt) or saline injection. Protein was precipitated with methanol, the supernatant evaporated to dryness and amino acids dissolved in distilled water. Concentration of alanine was determined with alanine dehydrogenase [20].

Hormones. In parallel experiments, aortic blood was drawn from AA and ANA rats 150 min after ethanol (1.5 g/kg body wt) or saline injection and the plasma used for the determination of insulin and glucagon. The hormones were measured by means of radioimmunoassay kits from International-CIS (Saint-Quentin-Yvelines C, France).

Statistics. Comparisons between the two rat lines were made by Student's *t*-test. The results are given as mean ± SEM.

RESULTS

The rate of alcohol elimination in the AA and ANA rats after the intraperitoneal ethanol dose was almost identical (Fig. 1), which is in accordance with previously published results [24]. At the timepoint when the rate of gluconeogenesis and hepatic glycogen content were measured, i.e. 150 min after alcohol administration, the blood alcohol concentration was 15.9 ± 1.4 mM in the AA rats and 15.3 ± 0.9 mM in the ANA rats.

Alone the handling of the animals and the injection of saline increased the blood glucose level (Table 1). Although such a stress effect has sometimes been mentioned, only a few [25] have reported as marked an effect as found in this study. Administration of alcohol to the animals increased the blood glucose level significantly in the ANA rats, while in the AA rats the increase was smaller and did not reach the level of statistical significance (Table 1). The hyperglycaemic effect of ethanol in fed animals has been reported earlier by others [25–28] but not everybody has been able to demonstrate it [29]. The rate of glucose synthesis was measured in fed rats, since it is in this state that voluntary alcohol consumption is measured and the differences in the alcohol-drinking behaviour of the AA and ANA rats seen. In the absence of ethanol, the concentration of radioactive glucose was similar in the plasma of both the AA and ANA rats (Table 2). Since there are no interstrain differences in the liver-to-body weight ratios between these rats [15], this was taken as an indication that the rats of the two strains synthesised glucose from alanine at the same rate (Table 2). Ethanol had no significant effect on the rate of gluconeogenesis in the ANA rats whereas in the AA rats a significant

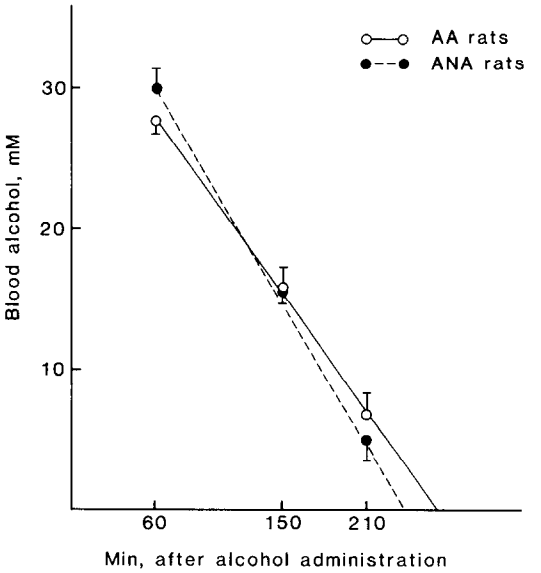

Fig. 1. Blood ethanol concentrations in AA and ANA rats after intraperitoneal administration. Blood samples were taken from tail at the indicated timepoints after an ethanol dose of 1.5 g/kg body weight. The number of animals in each group was 9.

Table 1. Effect of ethanol on blood glucose concentration in AA and ANA rats

Time (hr)	Blood glucose (mmol/l)			
	AA rats		ANA rats	
	Saline	Ethanol	Saline	Ethanol
0	5.24 ± 0.10	5.63 ± 0.18	5.24 ± 0.16	5.65 ± 0.37
1	5.80 ± 0.08***	6.43 ± 0.44	5.52 ± 0.20	6.21 ± 0.34
2	5.71 ± 0.14*	6.27 ± 0.43	5.71 ± 0.30	6.56 ± 0.41
3	5.73 ± 0.20*	6.39 ± 0.53	5.51 ± 0.27	6.50 ± 0.34†

Concentration of blood glucose was measured at indicated timepoints after an i.p. injection of ethanol (1.5 g/kg) or an equal volume of saline. The results are means ± SEM of 9 animals. * Significantly different from the control value (time 0), $P < 0.05$. *** Significantly different from the control value (time 0), $P < 0.001$. † Significantly different from the corresponding saline-group (time 3 hr), $P < 0.05$.

Table 2. Effect of ethanol on the rate of gluconeogenesis from alanine in AA and ANA rats

Treatment	Gluconeogenesis (nmol ^{14}C-labeled glucose formed/5 min × ml plasma)		Interstrain difference
	AA	ANA	
Saline	170 ± 39	188 ± 47	ns
Ethanol	320 ± 27**	211 ± 30	$P < 0.05$

^{14}C-labelled alanine was injected into the portal vein 150 min after an i.p. injection of ethanol (1.5 g/kg) or saline. Exactly 5 min later the amount of radioactivity incorporated into glucose was measured from a blood sample taken from the aortic bifurcation. The results are means ± SEM of 9 animals. ** Significantly different from the saline-treated group, $P < 0.01$. ns = not significant.

increase was observed. In the presence of ethanol the difference between the two rat strains was statistically significant. This finding supports the view that gluconeogenesis may be physiologically important even in fed animals as previously demonstrated by Park *et al.* in studies on dietary carbohydrate [30].

For saline-treated animals, plasma alanine concentration was significantly higher in the ANA rats than in the AA rats (Table 3). Ethanol decreased the alanine concentration in both rat strains; the decrease was more profound in the ANA strain, which caused the strain difference to disappear. Hepatic alanine concentration was slightly higher in the ANA rats than in the AAs and the decreasing effect of ethanol was similar to that found in plasma in this study and earlier in perfused liver [31]. As the concentration of alanine undergoes parallel changes in plasma and liver of both AA and ANA rats, it seems that supply of alanine, which in the physiological concentration range is limited by the rate of translocation across the plasma membrane [32], is not the reason for the observed difference in the rate of gluconeogenesis.

In an attempt to explain the observed differences in gluconeogenesis, and also because it has been suggested previously that the animals with high voluntary alcohol intake are prediabetic [3–5], we measured concentrations of insulin and glucagon in the plasma of the AA and ANA rats. When rats were given saline, there was no strain difference

Table 3. Effect of ethanol on plasma and liver alanine concentrations in AA and ANA rats

Tissue	Treatment	AA	ANA	Interstrain difference
Plasma (μmol/l)	Saline	250 ± 14	334 ± 20	$P < 0.01$
	Ethanol	208 ± 14*	193 ± 15***	ns
Liver (μmol/g)	Saline	2.28 ± 0.44	2.83 ± 0.26	ns
	Ethanol	0.23 ± 0.04***	0.30 ± 0.09***	ns

Alanine concentration was measured in aortic blood and liver 150 min after ethanol (1.5 g/kg body wt) or saline injection. The results are means ± SEM of 9 animals. * Significantly different from the saline-treated group, $P < 0.05$. *** Significantly different from the saline-treated group, $P < 0.001$. ns = not significant.

Table 4. Effect of ethanol on concentrations of insulin and glucagon in AA and ANA rats

Hormone (nmol/l)	Treatment	AA (n = 6)	ANA (n = 11)	Interstrain difference
Glucagon	Saline	0.24 ± 0.03	0.21 ± 0.02	ns
	Ethanol	0.26 ± 0.01	0.24 ± 0.02	ns
Insulin	Saline	0.16 ± 0.01	0.14 ± 0.02	ns
	Ethanol	0.14 ± 0.03	0.20 ± 0.02*	ns
Glucagon/ insulin	Saline	1.55 ± 0.26	1.58 ± 0.16	ns
	Ethanol	1.98 ± 0.28	1.26 ± 0.16	$P < 0.05$

Hormone concentrations were determined from aortic blood 150 min after an intraperitoneal injection of ethanol (1.5 g/kg) or a corresponding volume of saline. The number of animals is given in parenthesis. * Significantly different from the saline-treated group, $P < 0.05$. ns = not significant.

in the plasma concentration of insulin or glucagon (Table 4). Ethanol had no effect on the concentrations of these hormones in the AA rats, whereas in the ANA rats the insulin concentration increased significantly and glucagon concentration remained unaltered. The glucagon/insulin ratio, which has been shown to be more important in the regulation of metabolism than the actual concentration of either hormone [33], was significantly lower after ethanol treatment in the ANA rats than in the AA rats. According to Parrilla *et al.* [33] such a change could only alter the rate of glycogenolysis and not that of gluconeogenesis.

The hepatic glycogen content was significantly higher after saline treatment in the ANA rats than in the AA rats (Table 5). Alcohol administration had no effect on the glycogen stores in the AA strain but decreased them in the ANA strain.

DISCUSSION

At the time when the effect of ethanol on gluconeogenesis was measured, the mean blood alcohol concentration was about 15 mM in both the AA and ANA rats. Such a high alcohol level is seldom reached when the rats are able to freely choose the amount of alcohol ingested [24, 34]. The mean blood alcohol concentration after a peak drinking period has been found to range from 5.9 to 9.3 mM in the AA rats and to be about 0.9 mM in the ANA animals [34]. In experiments with dogs, Lochner *et al.* [35] observed that an alcohol concentration of 2–4 mM decreased hepatic glucose output by 65%. Guynn and Pielik [36] found that an alcohol concentration of 1 mM significantly altered the metabolite concentrations and the redox state in perfused rat liver. Rat liver alcohol dehydrogenase has a K_m value of 0.59–2.1 mM [37] depending on the conditions of measurement. It may, therefore, be assumed that many reactions affected by the alcohol oxidation show a response at alcohol concentrations at or even below the K_m level.

Table 5. Effect of ethanol on hepatic glycogen content in AA and ANA rats

Strain	Treatment	Glycogen mg/g liver	Interstrain difference
AA	Saline	38.9 ± 1.6	
	Ethanol	38.8 ± 4.1	
ANA	Saline	54.6 ± 5.6	$P < 0.05$
	Ethanol	44.9 ± 6.3	ns

Hepatic glycogen content was measured 150 min after an intraperitoneal injection of ethanol (1.5 g/kg) or a corresponding volume of saline. Results are means ± SEM of 9 animals. ns = not significant.

Alcohol lowers the blood glucose level in fasted animals, but an increase has been reported in fed animals and in man [25, 38–40]. Both the breakdown of hepatic glycogen and the formation of glucose by gluconeogenesis have been reported to increase, possibly contributing to the increase in the blood glucose concentration. Furthermore, the rate of glucose utilisation in extrahepatic tissues is decreased in the presence of alcohol [41], which may also affect the blood glucose level.

In the present study, ethanol produced only a slight increase in blood glucose concentration in the alcohol-preferring, AA rats and a somewhat larger increase in the alcohol-avoiding, ANA rats (Table 1). Although the change was unidirectional in the two strains, the mechanism for the maintenance and increase of blood glucose concentration seems, according to results shown in Tables 2 and 5, to be different. To elucidate this difference, the total amount of glucose formed in glycogenolysis and gluconeogenesis during the 150 min experimental period was calculated (Table 6). It is evident that in the ANA rats gluconeogenesis and glycogen breakdown contribute equally to the increase of the blood sugar level. The AA rats, on the other hand, appear to rely only on gluconeogenesis, the rate of which they are able to increase in the presence of ethanol. Based on this result, it can be assumed that long-term alcohol consumption would deplete the glycogen stores of ANA rats much faster than those of AA rats. It is tempting to speculate whether a total depletion of hepatic glycogen would eventually lead to hypoglycaemia in ANA rats, and possibly altered drinking behaviour.

It is conceivable that ethanol-induced changes of carbohydrate metabolism may be counteracted by hormonal regulatory mechanisms. It can also be hypothesised that inborn hormonal balance or the effect of alcohol and/or its metabolites on hormone secretion might modify voluntary alcochol consumption. In this study, however, the levels of insulin and glucagon which are the main carbohydrate-regu-

Table 6. Relative contribution of gluconeogenesis and glycogenolysis to glucose production in the presence of ethanol

Rat strain	Glucose production (μmol/g liver × 150 min)		% of total	
	Glycogenolysis	Gluconeogenesis	Glycogenolysis	Gluconeogenesis
ANA	48.5	58.8	45.2	54.8
AA	0.5	89.2	0.6	94.4

The change in glycogen content, calculated from the results in Table 5, was converted to glucose equivalents as in Van Handel [23]. For the calculation of gluconeogenesis per g liver, it was assumed that the newly formed glucose was distributed evenly in extracellular water; extracellular water volume was assumed to be 30% of body weight [22] and the liver to body weight ratio 3.23% [42].

lating hormones were very similar in the two strains when saline was administered. Alcohol had a significant effect only on the plasma insulin level of the ANA rats, which in the presence of glucagon cannot explain the lower rate of gluconeogenesis in these animals. Whether there are inborn differences regarding other hormones, remains to be studied.

A general conclusion from the present study is that nondiabetic AA and ANA rats in the fed state employ different mechanisms to maintain their carbohydrate homeostatis after alcohol ingestion. Gluconeogenesis, which seems to be the mechanism in the AA rats, is in the long run much more efficient than glycogenolysis, by which the ANA animals partly regulate their blood glucose level. Further research is, however, needed to clarify the effect of these differences in glucose metabolism on alcohol drinking behaviour in AA and ANA rats.

Acknowledgements—We would like to thank Ms Riitta Turtiainen and Ms Marja Hirvonen for excellent technical assistance. This work was in part supported by a research grant to A. R. P. from the Finnish Foundation for Alcohol Studies.

REFERENCES

1. K. Eriksson, *Ann. N.Y. Acad. Sci.* **197**, 32 (1972).
2. T.-K. Li, L. Lumeng, W. J. McBride and J. M. Murphy, *Alcohol Alcoholism*, Suppl. 1, 91 (1987).
3. J. A. Goas, R. W. Pelham and A. S. Lippa, *Pharmac. Biochem. Behav.* **10**, 557 (1979).
4. M.-E. Quintanella, L. Tampier and J. Mardones, *IRCS J. Med. Sci.* **10**, 534 (1982).
5. D. M. Connelly, J. M. Unwin and P. V. Tabernes, *Biochem. Pharmac.* **32**, 221 (1983).
6. B. Lindegård and M. Hillbom, *Acta Neurol. Scand.* **75**, 195 (1987).
7. K. Furuno, M. Arakana, A. Shino and Z. Suzuoki, *J. Nutr.* **105**, 1253 (1975).
8. M. E. Hillbom, *Acta Pharmac. Toxic.* **29**, 95 (1971).
9. O. A. Forsander, M. E. Hillbom and J. D. Sinclair, *Substance and Alcohol Actions/Misuse* **5**, 193 (1984).
10. N. Freinkel, D. L. Singer, R. A. Arky, S. J. Bleicher, J. B. Anderson and C. K. Silbert, *J. clin. Invest.* **42**, 1112 (1963).
11. N. Freinkel, A. K. Cohen, R. A. Arky and A. F. Foster, *J. clin. Endocrinol. Metab.* **25**, 76 (1965).
12. R. A. Arky, in *Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), Vol. 1, pp. 199–227. Plenum Press, New York (1971).
13. H. Ichii, F. Okune, J.-G. Joly and M. Tsuchiya, *Leber Magen Darm* **8**, 247 (1978).
14. O. A. Forsander and A. R. Pösö, *Alcohol Alcoholism* **21**, A35 (1986).
15. C. J. P. Eriksson, *Biochem. Pharmac.* **22**, 2283 (1973).
16. H. U. Bergmeyer and E. Bernt, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), Vol. 3, pp. 1205–1215. Academic Press, New York (1974).
17. M. S. Ayuso-Parrilla, A. Martin, P. Vega, T. Girbes and R. Parrilla, in *Recent advances in Obesity and Diabetes Research* (Eds. N. Melchiorda, D. L. Horwitz and P. V. Tabern), pp. 227–238. Raven Press, New York (1984).
18. L. E. Mallette, J. H. Exton and C. R. Park, *J. biol. Chem.* **244**, 5713 (1969).
19. G. E. Mortimore and C. E. Mondon, *J. biol. Chem.* **245**, 2375 (1970).
20. D. H. Williamson, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), Vol. 3, pp. 1679–1682. Academic Press, New York (1974).
21. A. K. Groen, H. J. Sips, R. C. Vervoorn and J. M. Tager, *Eur. J. Biochem.* **122**, 87 (1982).
22. B. Friedman, E. H. Goodman, Jr. and S. Weinhouse, *J. biol. Chem.* **242**, 3620 (1967).
23. E. Van Handel, *Analyt. Biochem.* **11**, 256 (1965).
24. K. Eriksson, *The Finnish Foundation for Alcohol Studies* **20**, 121 (1972).
25. C. Hollstedt, *Opuscula Medica*, Suppl. LII (1981).
26. D. M. Tennent, *Quart. J. Stud. Alc.* **2**, 271 (1941).
27. O. K. Vartia, O. A. Forsander and F.-E. Krusius, *Quart. J. Stud. Alc.* **21**, 597 (1960).
28. E. S. Perman, *Acta Physiol. Scand.* **55**, 189 (1962).
29. V. Marks, *Clinics Endocrinol. Metab.* **7**, 333 (1978).
30. J. J. Y. Park, C. D. Berdanier, O. E. Deaver, Jr. and B. Szepesi, *J. Nutr.* **116**, 1193 (1986).
31. A. R. Pösö, C. A. Surmacz and G. E. Mortimore, *Biochem. J.* **242**, 459 (1987).
32. H. J. Sips, A. K. Groen and J. M. Tager, *FEBS Lett.* **119**, 271 (1980).
33. R. Parrilla, M. N. Goodman and C. J. Toews, *Diabetes* **23**, 725 (1974).
34. J. Aalto, *Alcohol* **3**, 73 (1986).
35. A. Lochner, J. Wulff and L. L. Madison, *Metabolism* **16**, 1 (1967).
36. R. W. Guynn and J. R. Pielik, *J. clin. Invest.* **56**, 1411 (1975).
37. H. Theorell and R. Bonnichsen, *Acta Chem. Scand* **5**, 1105 (1951).
38. D. L. Topping, D. G. Clark, R. J. Illman and R. P. Trimble, *Horm. Metab. Res.* **14**, 361 (1982).
39. H. A. Krebs, R. A. Freedland, R. Hems and M. Stubbs, *Biochem. J.* **112**, 117 (1969).
40. O. Forsander, K. O. Vartia and F.-E. Krusius, *Med. Exp. Fenn.* **36**, 1 (1958).
41. H. Yki-Järvinen and E. A. Nikkilä, *J. Clin. Endocrinol. Metab.* **61**, 941 (1985).
42. A. R. Pösö and M. E. Hillbom, *Biochem. Pharmac.* **26**, 331 (1977).