Biochemical Pharmacology, Vol. 28, pp. 969–974.

0006-2952/79/0401-0969 $02.00/0

ROLE OF CARNITINE IN PROMOTING THE EFFECT OF ANTIDIABETIC BIGUANIDES ON HEPATIC KETOGENESIS*

ATTILA SANDOR, JANOS KERNER and ISTVAN ALKONYI

Biochemical Institute, University Medical School, Pécs, Hungary

(*Received* 27 *June* 1978; *accepted* 16 *October* 1978)

Abstract—Effects of biguanides on carnitine content of rat and guinea pig liver and on capacity of rat liver slices for ketogenesis were studied. In acute experiments, fed, 24-hour and 48-hour fasted male rats were given a single dose of buformin and the carnitine and acetylcarnitine level in the tissues were determined 1 or 3 hr afterwards. The same was performed on fed guinea pigs. In all the 1-hr groups we found an increase ranging from 30 to 50 per cent in hepatic carnitine level. In chronic experiments rats were treated with buformin or metformin for 6 days. The carnitine content, carnitine acetyltransferase and carnitine palmitoyltransferase activities were determined. The respective carnitine levels in the buformin- and metformin-treated groups were 4 times and 2.5 times the control value expressed on a per gram basis. In addition, carnitine acetyltransferase activity, given as mU/mg mitochondrial protein, increased 2-fold in the buformin-treated group. The increase in carnitine content strongly suggests that liver has enhanced capacity for oxidation of fatty acids and consequently for production of ketone bodies. The latter has been verified in the chronic experiments by the following observations: (1) The buformin administration increased the total ketone body content of the freeze-clamped liver specimens to 210 per cent of the control value. The calculated mitochondrial $NAD^+/NADH$ ratio was reduced from 10.6 to 5.96 in the same specimens. (2) the liver slices from treated animals formed 30–40 per cent more ketone bodies than those from control ones during the 30-min and 60-min incubations. (3) The ketone body associated radioactivity deriving from Na-[1^{14}-C]palmitate accounted for 90.5 per cent of water soluble radioactivity in slices from treated animals, whereas it accounted for 66.8 per cent in slices from control ones.

Most of the described effects of antidiabetic biguanides refer, as a matter of course, to the carbohydrate metabolism. The mutual influence of carbohydrate and lipid combustion on each other is elucidated in many details and often termed as the glucose/fatty acid cycle [1]. This interaction raises the issue that investigation on the lipid side may reveal primary points of attack, which may elicit, in turn, some known drug effects on carbohydrate metabolism [2]. On the other hand, an effect on the fate of fatty acids may also be secondary to a change produced primarily in glucose metabolism. Investigating the changes in lipid metabolism, we paid primary attention to those related to carnitine. Of the steps of fatty acid oxidation the reaction catalised by carnitine palmitoyltransferase (CPT†, EC2.3.1.21) plays a key role. This proposal of Fritz [3] comes from his original observation [4] and has been confirmed by many authors. The CPT reaction is shown to be involved in the transport of activated fatty acids from the cytosol into the mitochondrial matrix across the inner membrane barrier. Moreover, it is the level of carnitine substrate that is decisive in limiting the rate of CPT reaction and fatty acid oxidation, as appears from recent reports [5–7].

The other well-characterized enzyme in this field is the carnitine acetyltransferase (CAT, EC 2.3.1.7). Its role in the cell metabolism is not quite clear, but it probably participates in buffering the "acetyl-pressure" [8]. Considering the above, we have supposed that, if the biguanides change lipid metabolism either directly or indirectly, they affect the activity of enzymes related to carnitine and/or the level of carnitine in the tissues. In a previous study we tested whether biguanides inhibit the CPT enzyme. By reducing fatty acid oxidation, this inhibition would allow enhanced glucose utilization in accordance with the glucose/fatty acid cycle. However, we failed to point out any inhibition on solubilized CPT [9] or on membrane-bound CPT (unpublished results). In the present paper the carnitine level and activity of related enzymes have been studied *in vivo* under biguanide influence. The noteworthy change we have found is a rise in quantity of hepatic carnitine. This finding has led us to the presumption that biguanides accelerate fatty acid oxidation and ketone body production in the liver; this will also be supported in this work.

MATERIALS AND METHODS

Animals and treatment. Male Sprague–Dawley rats (200–220 g) and young male guinea pigs were housed in light-controlled room at 25° and fed carbohydrate-rich laboratory chow containing all necessary vitamins and minerals. In acute experiments, fed, 24-hour and 48-hour fasted rats and fed guinea pigs were given a single dose of buformin intraperitoneally and killed 1 hr and 3 hr afterwards. The applied dose was 25 mg/kg for guinea pigs and 50 mg/kg for rats. In chronic experiments rats were treated subcutaneously with buformin (50 mg/kg/day) or metformin (100 mg/kg/day) for 6 days. They were killed 3 hr after the last dose.

All animals were sacrificed between 9.00 and

* A preliminary report of this work was presented at the Proceedings of Conference of the Hungarian Biophysical and Biochemical Societies, Pécs, July 1977.

† Abbreviations: CPT = carnitine palmitoyltransferase; CAT = carnitine acetyltransferase; DTNB = 5,5′-dithiobis-2-nitrobenzoic acid.

11.00 a.m. After decapitation a portion of liver was freeze-clamped [10] within 10 sec and excised for metabolite determinations. In chronic experiments the whole liver was removed for preparing mitochondria and slices.

Metabolite determinations. A 150–200 mg portion of liver was extracted with 1.5–2.0 ml of 0.5 N perchloric acid in a hand-driven conic glass homogenizer. An aliquot of the neutralized extract was analysed for acetylcarnitine as previously described [11]. Ketone bodies* were determined in the same extract and in the extract of incubated liver slices (see below) as previously described [12, 13]. Total carnitine† was determined as free carnitine by the DTNB-method after alkaline hydrolysis of all carnitine esters [11]. (At the same time the alkaline hydrolysis reduced the blank extinction caused by thiol-compounds to a minimum.) The recovery of extraction and determination was estimated by addition of tracer DL-[methyl-^{3}H]carnitine and authentic L-carnitine and was found to be above 95 per cent.

Enzyme activities. Liver was homogenized in 10 times (w/v) the volume of 0.25 M sucrose, 50 mM Tris. HCl pH 7.5, 1 mM ETDA and mitochondria were isolated by conventional centrifugation at 5°. The pellet from 1 g original tissue was suspended in 2 ml of cold Triethanolamine. HCl pH 7.4, 1 mM ETDA supplemented with Triton X-100 to 0.1% and CAT and CPT were assayed in this solution.

CAT enzyme was assayed by two independent methods representing both directions of the reaction. One method measures the released CoASH by thiol reagent DTNB [14], while the other method measures the formed acetyl–CoA via coupled optical test [15]. The reactions were monitored by a Specord UV VIS recording spectrophotometer. The activities were expressed as mU/mg mitochondrial protein.

The activity of CPT was assayed by a radioactive exchange method, measuring the incorporation of L-[methyl-^{3}H]carnitine into (−) palmitoylcarnitine [15]. The activity was expressed in arbitrary units, as c.p.m./mg protein/minute.

Incubation of liver slices. The slices of 100 mg were cut from the liver by a special tool having two paralleled razor blades. Incubation was done in 25-ml Erlenmayer flask at 37°, with shaking. Calcium and bicarbonate-free Krebs–Henseleit solution buffered by 0.03 pH 7.4 HEPES and supplemented with 1% bovine albumin served as medium [7]. The incubation was initiated by adding 2 ml of the medium after rinsing the flask with O_2, then 30 or 60 min later the reaction was arrested by addition of 0.5 ml 2.5 N perchloric acid. Ketone bodies were determined as mentioned. Two liver slices were used from each animal.

Experiments with Na-[1-^{14}C] *palmitate.* The tissue slice was incubated in the above mentioned medium supplemented with 0.25 mM, approx. 1 μCi Na-[1-^{14}C]palmitate complexed with bovine serum albumin. The flask was equipped in the center well with a small removable vial containing 100 μl 10% NaOH. Otherwise the conditions were the same as detailed above. After stopping the incubation by injecting perchloric acid through the rubber cap, the shaking was continued for 1 hr at 25°. The small vial was then transferred into a counting vial. All sets of incubations included two flasks without slices. In the neutralized perchloric acid extract the following measurements were performed:

(a). Concentration of both ketone bodies by enzymatic analysis.

(b). Water-soluble radioactivity, i.e., the activity of the extract, which represents the oxidized products of Na-[1-^{14}C] palmitate. The aliquots were backwashed with equal volume of n-butanol before counting.

(c). Ketone body associated radioactivity (a part of the water-soluble radioactivity). Activity caused by [1-^{14}C] acetoacetate was identified as $^{14}CO_2$ liberated by aniline citrate [17]. Since the ratio of labelling in the carboxyl group (1-^{14}C) to the labelling in the acetone moiety (3-^{14}C) is close to unity [18], we doubled the 1-^{14}C activity and obtained the activity of acetoacetate. The total activity of both ketone bodies was calculated from the data of enzymatic analysis on the basis that the specific activity of the two ketone bodies is strictly equal [19].

All radioactive samples were measured in Bray's solution by a Beckman LS-230 counter.

Materials. CoA, NAD, NADH and necessary enzymes for analysis were obtained from Boehringer, Mannheim. Bovine serum albumin (fatty acid free) was from Sigma Chemical Co., St Louis, MO. L-carnitine was from Koch-Light, Colnbrook, Bucks. DL-[methyl-^{3}H]carnitine and [1-^{14}C]palmitic acid were purchased from the Radiochemical Centre, Amersham. Acetyl–CoA [20], (−) acetylcarnitine [21] and (−) palmitoylcarnitine [22] were prepared as described. Buformin (butyl-biguanide) and metformin (dimethyl-biguanide) were gifts from Chinoin, Budapest, and Aron, Suresnes, respectively.

Statistics. Values refer to means ± S.E.M., the numbers in parentheses indicate animals used. Student's *t* test was used for statistical analysis.

RESULTS

Acute effects of buformin. The effect of a single dose

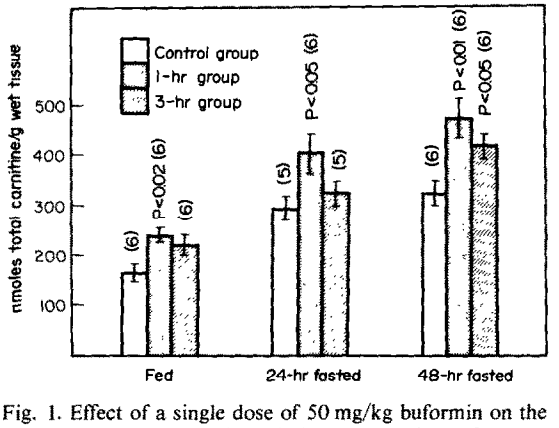

Fig. 1. Effect of a single dose of 50 mg/kg buformin on the carnitine content of rat liver. Animals were killed after 1 or 3 hr of intraperitoneal injection of the drug and total carnitine was determined as described in Materials and Methods. Each column represents the mean ± S.E.M. for the number of animals shown in parentheses.

* "Ketone bodies" or "total ketone bodies" refer to the sum of 3-OH-butyrate and acetoacetate, throughout the text.

† "Carnitine" or "total carnitine" refer to L(−)carnitine measured after alkaline hydrolysis, i.e., the sum of free and esterified carnitine, throughout the text.

of buformin on hepatic carnitine content in fed, 24-hr and 48-hr fasted rats is shown in Fig. 1. Starvation is accompanied by accumulation of carnitine in the liver [6, 23], as is also seen by comparing the control groups of Fig. 1. Biguanides have long been known to inhibit intestinal glucose absorption [24], so they can produce a starvation-like effect. Thus, to elucidate the mechanism whereby they elevate hepatic carnitine level, it was of importance to involve fasted animals. As appears from Fig. 1, buformin can also exert a carnitine-increasing effect in liver of fasted animals, where the inhibition of glucose absorption surely does not operate.

To show that the above effect of buformin is not restricted to rats, we performed a set of experiments on guinea pigs. Figure 2 shows that buformin can also raise the hepatic carnitine level in guinea pigs. Additionally, a decrease in the acetylcarnitine/carnitine ratio was observed. This decrease may be related to the extreme sensitivity of this species to biguanides. Unlike rats, the employed dose (25 mg/kg) lowers hepatic ATP and normal blood sugar level in guinea pigs [25].

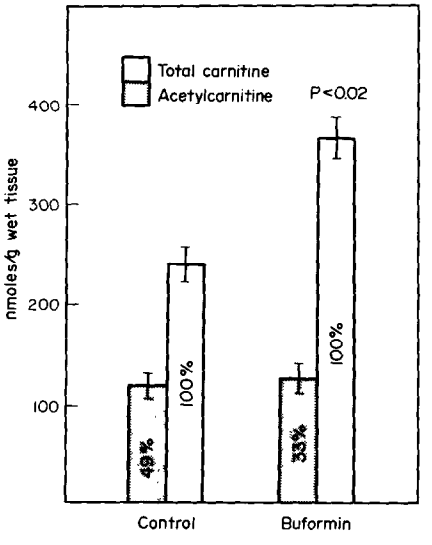

Fig. 2. Effect of a single dose of 25 mg/kg buformin on the acetylcarnitine and total carnitine content of guinea pig liver. Animals were killed 1 hr after the intraperitoneal injection of the drug and the determinations were performed as described in Materials and Methods. Columns represent the means ± S.E.M. for six animals.

Effects of chronic biguanide administration. As can be seen from Table 1, the carnitine level shows a striking increase, while the CPT activity is unchanged in the liver after 6-day administration of biguanides. Starvation, alloxan-induced diabetes, glucagon and anti-insulin serum infusion are shown to elevate both hepatic ketogenesis and hepatic carnitine concentration in rats. It is of major importance that the resulting carnitine level is proportional to the capacity of liver for ketogenesis [6, 26]. In view of these data, it is reasonable to study the ketogenic capacity of liver slices from biguanide-treated animals.

Further, we observed a two-fold increase in CAT activity (Table 1). At present, the significance of this finding is not clear. It may be of interest to remember the effect of hypolipidaemic clofibrate, which evokes a 30-fold increase (approx.) in rat liver CAT activity [27]. (The role of this effect in the mechanism of action of clofibrate is also unknown.)

We should remark that the liver weight in the animals treated for 6 days decreased to 60 per cent of the control value, while the normalized liver weight (g liver/100 g body wt) remained unchanged (data/not shown). It is also noteworthy that the carnitine level in the skeletal muscles was not affected both in acute and chronic experiments (data not shown).

Hepatic ketone body level and ketone body production by liver slices. Determination of ketone body content was necessary for calculation of the net ketone body production by liver slices. To preserve the 3-OH-butyrate/acetoacetate ratio existing in the liver, we obtained the specimens by freeze-clamp method. The buformin treatment evokes a striking rise in total ketone body level, which is evidence for enhanced fatty acid oxidation (Table 2). The observed increase in the 3-OH butyrate/acetoacetate ratio which reflects a negative shift of the mitochondrial $NAD^+/NADH$ redox potential, is also indicative of increased fatty acid oxidation [29]. This is consistent with the abovementioned starvation-like effect of biguanides. The other plausible explanation for this negative shift would be the inhibition of oxidative phosphorylation. This effect of biguanides is well documented under *in vitro* conditions [30, 31]. However, buformin is a less potent (but

Table 1. Effect of chronic biguanide administration on carnitine content and carnitine acyltransferase activities in rat liver

Group	Carnitine nmoles/g liver	CAT in mU (per mg mitochondrial protein) A	B	CPT in c.p.m. × 10^{-3}.min^{-1}
Control (5)	210 ± 8.1	2.82 ± 0.11	3.74 ± 0.12	11.5 ± 0.82
Buformin (5)	897 ± 31.4	5.41 ± 0.32	5.66 ± 0.33	11.9 ± 0.91
Metformin (5)	440 ±25.2			

Animals were given subcutaneously 40 mg/kg/day buformin or 80 mg/kg/day metformin for 6 days. Total carnitine, carnitine acetyltransferase (CAT) and carnitine palmitoyltransferase (CPT) were assayed as described in Materials and Methods. CAT activity was assayed by DTNB method [14] in column A and by coupled Warburg optical test [15] in column B. Values give means ± S.E.M. for the number of animals shown in parentheses. Statistical significance is $P < 0.001$ for all cases except that of the unchanged CPT activity.

Table 2. Effect of chronic buformin administration on the concentration of ketone bodies in rat liver

	Control (5)	Buformin (5)
Total ketone body, nmoles/g wet liver	276.0 ± 25.2	582.0 ± 60.4g ($P < 0.01$)
(3-OH-butyrate)/ (Acetoacetate)	1.9 ± 0.21	3.4 ± 0.35 (P 0.01)
Mitochondrial $NAD^+/NADH$	10.6	5.96

Rats were given subcutaneously 50 mg/kg/day buformin for 6 days. Ketone bodies were determined from freeze-clamped liver portions as described in Materials and Methods. Mitochondrial $NAD^+/NADH$ ratio was calculated from ratio of 3-OH-butyrate/acetoacetate as described [28]. Values represent means ± S.E.M. for the number of animals shown in parentheses.

Table 3. Effect of buformin administration on the fate of Na[1-^{14}C]palmitate in rat liver slices

Product	[1-^{14}C]palmitate equivalents (nmoles/g liver/30 min) Control (4)	Buformin (4)	% of effect
CO_2	1.3 ± 0.08	1.4 ± 0.1	—
Water-soluble material	14.5 ± 1.2	16.9 ± 1.2	+16
Ketone bodies	9.7 ± 0.8	15.2 ± 1.5	+56 ($P < 0.02$)
$\frac{\text{Ketone bodies}}{\text{Water-soluble material}} \cdot 100$	66.8	90.5	

Rats were given subcutaneously 50 mg/kg/day buformin for 6 days. A pair of 100 mg liver slices from each animal was incubated and metabolites were determined as described in Materials and Methods. Medium contained 0.25 mM Na-[1-^{14}C]palmitate giving 5400 d.p.m./nmole specific activity. Values represent means ± S.E.M. for the number of animals shown in parentheses.

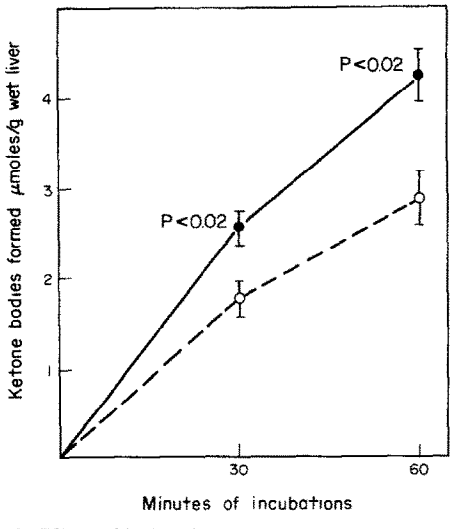

Fig. 3. Effect of buformin on ketone body formation of rat liver slices. Animals were given subcutaneously 50 mg/kg/day buformin for 6 days. Incubations were done in duplicate from each animal at each point. Incubation and determinations were performed as described in Materials and Methods. The original ketone body content (see Table 2) was subtracted. Points represent the means ± S.E.M. for four animals in both groups. ○— — —○ control, ●——● buformin-treated.

perhaps less dangerous) biguanide [31] and its hepatic tissue level is hardly up to that (>0.5 mM) required to inhibit oxidative phosporylation. (Buformin does not lower hepatic ATP and normal blood sugar level in rats [25]).

Incubated liver slices from buformin-treated rats show an increased capcity for ketogenesis (Fig. 3). Because of the very limited uptake by slices of exogenous fatty acids (see footnote to Table 3) fatty acids were omitted from the medium and purely the endogenous ketogenesis was studied.

Fate of Na-[1-^{14}C]*palmitate in liver slices.* The amount of oxidized radioactive palmitate (Table 3) can contribute a little to the net ketone body production by liver slices incubated under the same conditions (see Fig. 3). The labelled palmitate can therefore be assumed as tracer. It is seen (Table 3) that the participation of radioactive ketone bodies in the water-soluble radioactivity was increased by buformin treatment from 66.8 to 90.5 per cent.

DISCUSSION

Studying the lipid metabolism we have demonstrated that biguanides evoke enhanced ketogenesis with a concomitant carnitine enrichment in liver of intact animals. Regarding the mechanism whereby the drug brings about this effect, one of the possibilities is that

the increased fatty acid oxidation is secondary to the altered carbohydrate metabolism. In the field of carbohydrate metabolism, two known biguanide effects are closely related to ketogenesis: the inhibition of intestinal glucose absorbtion [24]; and the acceleration of glucose utilization (lactate production and oxidation) [25, 32 33]. (Both effects are demonstrated on intact animals or healthy subjects in the above references.) However they could alter ketogenesis in the opposite direction. Whereas the former could itself induce ketogenesis, the latter could act against ketogenesis (the lactate is a highly effective antiketogenic agent). Clearly, if the affected carbohydrate metabolism is responsible, the inhibition of glucose absorption is decisive; it overcomes the antiketogenic effect of lactate and results in the increased ketogenic capacity demonstrated here. The mechanism of developing carnitine enrichment in the liver under conditions of carbohydrate deprivation (e.g., starvation or impaired glucose absorption) and alloxan-induced diabetes in rats, has not been clarified in detail. To date, it has been assumed that carnitine is transported from the extrahepatic tissues into the liver, when the insulin/glucagon ratio in the serum decreases [6, 34].

The other possibility is that biguanides may increase hepatic carnitine level in a direct way, or more exactly, in a way other than by inhibition of glucose absorption, as it is suggested by the acute experiments (1-hr effect) on two species. Recently, it has been reported that the closest proportionality exists between ketogenic capacity and carnitine content of the liver when hepatic glycogen has been depleted [26]. Glycogen depletion is met by fasted animals, in which buformin evokes in 1 hr a significant rise in hepatic carnitine level. This rise strongly suggests, but does not prove, an enhanced ketogenesis.

Regardless of the mechanism, some conclusions can be drawn from the ketogenic effect of biguanides in intact animals. The possibility of increased fatty acid oxidation in liver has not been considered. In fact, a mechanism for biguanides, which is based on general and primary inhibition of fatty acid oxidation has been suggested [35]. Our results strongly challenge the suggestion that this mechanism operates in the liver. A recent report also casts a doubt on this mode of action in muscle [36].

Bigunanides have been shown to decrease hepatic lipid storage and to prevent steatosis in diabetes (the so-called anti-insulin effect) [37]. The increased fatty acid oxidation outlined above is consistent with this observation and lends it a molecular basis: carnitine-promoted oxidation of incoming fatty acids to ketone bodies.

Numerous studies with often conflicting conclusions have been published on the effect of biguanides on gluconeogenesis. Since enhanced ketogenesis is generally accompanied by enhanced gluconeogenesis, our finding indirectly supports the view that biguanides do not inhibit and may even accelerate gluconeogenesis in intact animals [38, 39] and in healthy subjects [32, 33, 40].

Supposing that the carnitine-accumulating effect of biguanides may also be produced in human therapy, one should keep some points in mind. Liver has been shown to have a high carnitine level (4–5 times the normal) in alloxan-induced diabetes in rats [6] and in sheep [41]. This experimental model may correspond to the insulin deficient human diabetes, for the therapy of which biguanides have not been indicated. Therefore, it does not necessarily follow from our findings that biguanides meet in the therapy an already high hepatic carnitine level and that they should increase it further. Biguanides are generally employed for the therapy of maturity-onset diabetes; however, we cannot conclude presently how the hepatic carnitine level may change in this type of diabetes.

REFERENCES

1. E. A. Newsholm and C. Start, *Regulation in Metabolism* p. 226. John Wiley and Sons, London (1973).
2. G. U. Corsisni, F. Sirigu, P. Tagliamonte and S. Muntoni, *Pharmac. Res. Commun.* **6**, 253 (1974).
3. G. Delisle and I. B. Fritz, *Proc. natn. Acad. Sci. U.S.A.* **58**, 790 (1967).
4. I. B. Fritz, *Acta physiol. scand.* **34**, 367 (1955).
5. J. D. McGarry and D. W. Foster, *J. biol. Chem.* **249**, 7984 (1974).
6. J. D. McGarry, C. Robles-Valdes and D. W. Foster, *Proc. natn. Acad. Sci. U.S.A.* **72**, 4385 (1975).
7. R. Christiansen, B. Borrebaek and J. Bremer, *FEBS Lett.* **62**, 313 (1976).
8. D. J. Pearson and P. K. Tubbs, *Biochem. J.* **105**, 953 (1967).
9. A. Sandor, J. Kerner and I. Alkonyi, *Acta Biochim. biophys. Acad. Sci. Hung.* **12**, 217 (1977).
10. A. Wollenberger, O. Ristan and G. Schoffa, *Pflüger's Arch. ges. Physiol.* **270**, 399 (1960).
11. D. J. Pearson, P. K. Tubbs and J. F. A. Chase, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmayer), 2nd. edn., p. 1758. Verlag Chemie, Weinheim (1974).
12. D. H. Williamson and J. Mellanby, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmayer), 2nd. edn., p. 1736. Verlag Chemie, Weinheim (1974).
13. J. Mellanby and D. H. Williamson, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmayer), 2nd. edn., p. 1740. Verlag Chemie, Weinheim (1974).
14. I. B. Fritz and S. K. Schultz, *J. biol. Chem.* **240**, 2118 (1965).
15. D. J. Pearson, J. F. A. Chase and P. K. Tubbs in *Methods in Enzymology* (Ed. J. M. Lowenstein) Vol. 14, pp. 612–622. Academic Press, New York (1969).
16. J. Bremer and K. R. Norum, *J. biol. Chem.* **242**, 1744 (1967).
17. J. A. Ontko, *Life Sci.* **3**, 573 (1964).
18. L. L. Chaikoff, D. S. Goldman, G. W. Brown, W. G. Dauben and M. Gee, *J. biol. Chem.* **190**, 229 (1951).
19. J. D. McGarry, M. J. Guest and D. W. Foster, *J. biol. Chem.* **245**, 4382 (1970).
20. E. R. Stadtman, in *Methods in Enzymology* (Eds S. P. Colowick and N. O. Kaplan), Vol. 3, p. 931. Academic Press, New York (1957).
21. S. Friedman and G. Fraenkel in Vitamins and Hormones (Eds R. S. Karris, G. F. Marrian and K. V. Thimann), Vol. 15, p. 85. Academic Press, New York (1957).
22. H. J. Ziegler, P. Bruckner and F. Binon, *J. org. Chem.* **32**, 3989 (1967).
23. J. Kondrup and N. Grunnet, *Biochem. J.* **132**, 373 (1973).
24. L. Biro, T. Banyasz, M. B. Kovacs and M. Bajor, *Klin. Wschr.* **39**, 760 (1961).
25. W. Losert, W. Kraaz, P. Jahn and A. Rilke, *Naunyn-Schmiedeberg's Arch. Pharmak.* **269**, 459 (1971).
26. C. Robles-Valdes, J. D. McGarry and D. W. Foster, *J. biol. Chem.* **251**, 6007 (1976).
27. M. T. Kahonen, *Biochim. biophys. Acta* **428**, 960 (1976).
28. D. H. Williamson, P. Lund and H. A. Krebs, *Biochem. J.* **103**, 514 (1967).

29. E. A. Newsholm and C. Start, *Regulation in Metabolism* p. 318. John Wiley and Sons, London (1973).
30. G. Schäfer, *Biochem. Pharmac.* **25**, 2005 (1976).
31. G. Schäfer, *Biochem. Pharmac.* **25**, 2015 (1976).
32. G. L. Searle, R. Gulli and R. R. Cavalieri, *Metabolism* **18**, 148 (1969).
33. R. A. Kreisberg, L. F. Pennington and B. R. Boshell *Diabetes* **19**, 53 (1970).
34. J. Bremer, *TIBS* **2**, 207 (1977).
35. S. Muntoni, in *Advances in Lipid Research* (Eds R. Paoletti and D. Kritchevsky) Vol. 12, pp. 311–377. Academic Press, New York (1974).
36. G. F. Tutwiler, *Res. Commun. Chem. Path. Pharmac.* **19**, 541 (1978).
37. J. Sterne, in *Oral Hypoglycemic Agents* (Ed. G. D. Campbell) pp. 193–245. Academic Press, New York (1969).
38. V. H. Davies, L. E. Martin, J. G. Mills, and C. J. Vardey, 7th Annu. Meet. Eur. Ass. Study Diabetes, Southampton, England. Abstr. No. 45 (1971).
39. H. D. Söling, *Acta Diabet. Lat.* **6**, Suppl. 1, 656 (1969).
40. H. Goschke, U. Kaufmann, H. Tholen and W. Berger, *Hormone Metab. Res.* **10**, 1 (1978).
41. A. M. Snoswell and G. D. Henderson, *Biochem. J.* **119**, 59 (1970).