The hepatic defect in glycogen synthesis in chronic diabetes involves the G-component of synthase phosphatase

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1. Hepatocytes from normal fed rats and from chronically (90 h) alloxan-diabetic rats were compared. The rate and the extent of activation of glycogen synthase in response to 60 mM-glucose were greatly decreased in diabetes. During incubation of gel-filtered extracts from broken hepatocytes, diabetes only decreased the rate of the activation, which became ultimately complete in either preparation. 2. Synthase phosphatase activity, as measured by the activation of purified hepatic synthase *b*, was decreased in chronic diabetes. The decrease was proportional to the severity of the diabetes, and reached 90% when the plasma glucose concentration was ≥ 55 mM. In contrast, phosphorylase phosphatase activity was not decreased. Synthase phosphatase activity was progressively restored by treatment with insulin for 20–68 h. 3. During the induction of diabetes and during insulin treatment there was a good correlation between the activity of synthase phosphatase and the maximal activation of synthase in glucose-stimulated hepatocytes from the same livers. 4. The decreased activity of synthase phosphatase in diabetes cannot be explained by an inhibitor. The decrease was much less marked when synthase phosphatase was assayed by the dephosphorylation of ^{32}P -labelled synthase from muscle. This observation suggested a loss of only one component of synthase phosphatase. 5. Cross-combination of subcellular fractions from control rats and from diabetic rats showed a preferential loss of G-component, with little or no loss of S-component. No G-component could be detected in severe diabetes. 6. The concentration of G-component is therefore of critical importance in the glucose-induced activation of glycogen synthase in the liver.

The response of the normal liver to a glucose load involves sequentially the inactivation of phosphorylase and the activation of glycogen synthase (Stalmans *et al.*, 1974a; Hue *et al.*, 1975). The inactivation of phosphorylase, and hence the inhibition of glycogenolysis, is initiated by the binding of glucose to phosphorylase *a*. The resulting change in conformation exposes the phosphorylated tail of the enzyme to the action of phosphorylase phosphatase (Stalmans *et al.*, 1974b; Madsen *et al.*, 1978). Removal of phosphorylase *a*, which exerts a strong inhibition on the activity of synthase phosphatase, allows the latter enzyme to convert glycogen synthase *b* into the active *a* form (Stalmans *et al.*, 1971). Synthase phosphatase activity in the liver stems from the co-operation of two proteins: a G-component that is tightly associated with glycogen particles, and an S-component that is present in the post-glycogen supernatant (Doperé *et al.*, 1980). Only the G-component is inhibited by phosphorylase *a* (Mvumbi *et al.*, 1983).

Several groups have reported that the activation of glycogen synthase and the accumulation of glycogen in response to glucose are impaired in isolated livers and hepatocytes from diabetic rats (Miller *et al.*, 1973; Whitton & Hems, 1975; Miller, 1978; Golden *et al.*, 1979). A more recent investigation showed an almost normal glycogenic response in hepatocytes from acutely (40 h) alloxan-diabetic rats (Bollen *et al.*, 1983). Thereafter an intrahepatic defect developed, which, in severely diabetic animals, became complete by the end of the third day after alloxan administration. The glucose-induced inactivation of phosphorylase still proceeded normally, but the ensuing activation of glycogen synthase was impaired. The quantitative importance of the latter defect correlated with the severity of the diabetic state.

The present paper describes an investigation into the nature of the hepatic lesion in chronic alloxan-diabetes. The defect appears to be explained by a loss of functional G-component.

Experimental

Materials, buffer and statistics

Purified glycogen synthase *b* from dog liver was a pool of the forms termed *b*₂ and *b*₃ by Doperé *et al.* (1980). Glycogen synthase *a* was purified from rabbit muscle essentially by method 2 of Nimmo *et al.* (1976). ³²P-labelled muscle synthase *b* was prepared by phosphorylation of the *a* form with different synthase kinases (see the legend to Fig. 3) as detailed by Mvumbi *et al.* (1983). ³²P-labelled phosphorylase *a* was prepared by phosphorylation of crystalline *b* enzyme from rabbit muscle (Yang *et al.*, 1980). Particulate glycogen was purified from dog liver by the phenol method (Laskov & Margoliash, 1963). Long-acting monocomponent insulin was a commercial pharmaceutical preparation (Lente, from Novo Industri). The source of other relevant products has been described (Doperé *et al.*, 1980; Bollen *et al.*, 1983).

Buffer A contained 0.25M-sucrose, 0.5mM-dithiothreitol and 50mM-imidazole, adjusted at room temperature to pH 7.4.

Results are expressed as means $\pm$ S.E.M. for the numbers of observations indicated in parentheses. Vertical bars in graphs indicate $\pm$ S.E.M. Statistical treatment of the data was by Student's *t* test for independent random samples, or by linear-regression analysis.

Handling of animals and livers

Animals. Male Wistar rats weighing about 250g and fed *ad libitum* were killed by decapitation, except when hepatocytes had to be isolated. About 0.5 ml of blood from the neck or from the vena cava was collected on 10 μ l of 0.2M-Na₂-EDTA and centrifuged. The rats were either untreated controls or were injected intravenously around 17:00h with alloxan monohydrate (45–52mg/kg body wt. in various experiments). Except for some data in Fig. 2, the diabetic animals were killed about 90h after alloxan injection; they were therefore 'chronically diabetic' as defined by Bollen *et al.* (1983). In each experiment the severity of the diabetes was assessed from the plasma glucose concentration at death; it was classified as 'moderate' (mean plasma glucose concentration about 40mM) or 'severe' (at least 55mM). 'Mildly' diabetic rats, with plasma glucose concentration around 25mM, were used in one experiment (Table 1). When indicated, 5 units of insulin were injected subcutaneously once daily at 13:00h, starting 92h after alloxan treatment (Fig. 2). The latter rats (*n* = 19) were moderately diabetic on the basis of the glucose concentration in blood taken from a sublingual incision 20h and 4h before the first insulin injection. They were killed 20h, 44h or 68h after the initiation of insulin treatment.

Hepatocytes. The preparation and incubation were as described by Bollen *et al.* (1983). The cells were incubated for 10min with 2mM-glucose, and then for a further 30min after the addition of 60mM-glucose. A steady-state concentration of glycogen synthase *a* was always attained at the end of the latter incubation (see Fig. 1a).

Homogenates and gel-filtered extracts. Livers were rinsed in ice-cold 0.15M-NaCl and homogenized in a Potter-Elvehjem tube in 4vol. of either cold buffer A (control rats) or buffer A plus 1% particulate liver glycogen (diabetic rats). In some experiments a liver lobe was ligated and removed from an anaesthetized rat before the start of the hepatocyte-isolation procedure. This lobe was homogenized as described above. In the experiments described in Fig. 1(b), 2ml of freshly prepared undiluted hepatocyte suspension (about 4×10^7 cells) was centrifuged at 8000g for 1–3s. The packed cells were resuspended in cold buffer A (control rats) or buffer A plus 1% particulate glycogen (diabetic rats) to a final volume of 2ml, and the suspensions were homogenized at 4°C in a Dounce tube fitted with a tight B pestle (50 strokes).

When required, the 20% homogenates of liver or hepatocytes were centrifuged in the cold for 10min at 8000g. Then 1ml of supernatant (extract) was filtered in the cold through a column (1.5cm $\times$ 5cm) of Sephadex G-25, equilibrated in buffer A. The protein filtrate was collected.

Assays

All enzyme activities were determined at 25°C. Glycogen synthase *a* and *a* + *b* were measured as described by Doperé *et al.* (1980); in hepatocyte preparations, the adaptation by Bollen *et al.* (1983) was used. One unit converts 1 μ mol of substrate into product/min in the appropriate assay conditions.

Glycogen synthase phosphatase was measured in dilute liver preparations by either the activation or the dephosphorylation of purified glycogen synthase. The assays were conducted as described by Mvumbi *et al.* (1983), in the presence of 3mM-AMP and 5mM-magnesium acetate to cancel the inhibitory effect of phosphorylase *a*. Unless otherwise stated, homogenates were assayed at a final concentration of 2%, and subcellular fractions at an equivalent dilution with respect to the liver tissue. One unit of synthase phosphatase converts 1 unit of hepatic synthase *b* into *a*/min.

Phosphorylase phosphatase activity was determined in gel-filtered liver extracts, with or without subsequent incubation with trypsin, by the rate of dephosphorylation of ³²P-labelled phosphorylase *a* (Doperé & Stalmans, 1982). The gel-filtered extracts were assayed at final concentrations of 2% and 0.1%, before and after treatment with trypsin

respectively. One unit of phosphorylase phosphatase dephosphorylates 1 unit of phosphorylase *a*/min.

Plasma glucose was measured by the glucose oxidase method (Dahlqvist, 1961).

Subcellular fractions of synthase phosphatase

The usual procedure for the separation of the S- and G-components of synthase phosphatase involves the use of glycogen-depleted livers from starved glucagon-treated rats (Doperé *et al.*, 1980). A cytosolic fraction, which contains all the synthase phosphatase activity, is prepared by high-speed centrifugation. After the addition of purified particulate glycogen, a second centrifugation separates the supernatant, which contains the S-component, from the glycogen-associated G-component. This procedure was adopted for the preparation of the components used to characterize different types of synthase *b* (Fig. 3).

A different separation procedure has allowed us to explore glycogen-containing livers also (Table 3). The livers were homogenized in a Potter-Elvehjem tube in 2 vol. of cold buffer A (control livers) or buffer A plus 1% particulate glycogen (diabetics). The homogenates were centrifuged for 10 min at 8000*g*, and the resulting extracts were centrifuged at 220 000*g* for 35 min. The high-speed pellet, consisting mainly of glycogen and microsomal fraction, was resuspended in buffer A to the

volume of the initial extract, and re-centrifuged at high speed. The supernatant (wash fluid) was combined with the first high-speed supernatant, as a source of S-component. The washed pellet containing the G-component was resuspended in buffer A (see Table 3).

Results

Activation of synthase in intact and broken hepatocytes

Fig. 1(a) illustrates the activation of glycogen synthase in hepatocytes in response to a massive glucose load (60 mM). Control hepatocytes rapidly attained a 90% conversion of synthase *b* into *a*, whereas in diabetic cells the conversion reached a plateau at 25%. Samples of the same cell preparations were homogenized, and gel-filtered cell extracts were incubated at 25°C (Fig. 1b). Full conversion of synthase *b* into *a* was achieved within 10 min in the control filtrate. The same ultimate result was obtained in the filtrate from diabetic cells, but it required 40 min. The initial rate of conversion was 4.5-fold lower in the filtrate from diabetic cells. In these same experiments a liver lobe was also removed before perfusion and homogenized. The activities of synthase phosphatase, measured in these dilute homogenates on purified hepatic synthase *b*, were 860 ± 70 and 142 ± 22 munits/g for

Fig. 1. *Effect of chronic diabetes on the activation of synthase in intact and in broken hepatocytes* Hepatocytes were isolated from the livers of three control rats and of three moderately diabetic rats. In (a) the hepatocytes were incubated at 37°C with the successive addition of 2 mM- and 60 mM-glucose (arrows). In (b) gel-filtered extracts of homogenized hepatocytes were incubated at 25°C in the presence of 3 mM-AMP and 5 mM-magnesium acetate. Synthase *a* and total synthase (which remained constant) were measured at the indicated time intervals.

Table 1. *Hepatic phosphoprotein phosphatase activities in mild and severe diabetes*

The activity of synthase phosphatase was measured with hepatic synthase *b* as substrate. The mean values for the control group are indicated in square brackets, in units/g of liver. Significantly different: * from the control group ($P < 0.002$); † from the mildly diabetic group ($P < 0.01$).

Condition (no. of animals)	Plasma glucose (mM)	Synthase phosphatase (% of control)	Phosphorylase phosphatase (% of control)	
			Basal	+ Trypsin
Control (12)	7.4 ± 0.6	100 ± 3 [0.98]	100 ± 5 [10]	100 ± 3 [242]
Mild diabetes (6)	25.8 ± 2.0*	35 ± 4*	113 ± 24	133 ± 8*
Severe diabetes (3)	56.6 ± 8.4*†	10 ± 2*†	331 ± 51*†	130 ± 9*

Table 2. *Absence of an inhibitor of synthase phosphatase from liver homogenates from severely diabetic rats*

Synthase phosphatase activity was measured in homogenates (final concn. 1%, w/v) on hepatic synthase *b*. Samples of 20% liver homogenates were heated for 4 min in a boiling-water bath. The heated samples were finely dispersed in a Dounce homogenizer, and centrifuged for 10 min at 3000g. Where indicated, the clear supernatants were added to the synthase phosphatase assay at a final concentration equivalent to a 5% homogenate. The plasma glucose concentration in the diabetic group was 57 ± 4 mM.

Homogenate	Supernatant from heated homogenate	Synthase phosphatase (relative activity)
Control (3)	—	100 ± 7
Diabetic (5)	—	22 ± 4
Control + diabetic (5)	—	125 ± 4
Control	Control (6)	69 ± 2
Control	Diabetic (6)	64 ± 1

the controls and the diabetic livers respectively (results not illustrated in Fig. 1).

Activities of phosphoprotein phosphatases

Decrease and restoration of synthase phosphatase.

Chronically diabetic rats were selected for the presence of either a mild or a severe type of diabetes, according to the plasma glucose concentration at death (Table 1). The decrease in synthase phosphatase activity (measured on hepatic synthase *b*) was clearly related to the severity of the diabetic state. Fig. 2 summarizes all our data on the relationship between synthase phosphatase activity in a dilute liver homogenate and the maximal activation of glycogen synthase (at 60 mM-glucose) in hepatocytes isolated from the same liver. The diabetic animals include rats with chronic diabetes (72 h or 90 h after alloxan injection) of various degrees of severity, as well as some rats examined 40 h or 52 h after alloxan injection. Also included are animals with chronic diabetes of moderate severity that were treated with insulin. No effect on either parameter was noted 20 h after the onset of treatment; for both parameters, the recovery was partial after 44 h, and essentially complete after 68 h (results not shown).

Phosphorylase phosphatase (Table 1). 'Total' phosphorylase phosphatase activity (after incuba-

tion with trypsin) was somewhat increased by the diabetic state, but irrespective of its severity. The directly assayable activity was unchanged in mild diabetes, but it was apparently increased over 3-fold in severe chronic diabetes (see discussion).

Nature of the decreased synthase phosphatase activity

Presence of an inhibitor? Dilute liver homogenates from control rats and from severely diabetic animals were assayed for synthase phosphatase activity on hepatic synthase *b*, either alone or combined (Table 2). The measured activity on combination was close to the value expected from arithmetic addition. The presence of a heat-stable inhibitor was also explored. The heat-stable fraction from diabetic livers was somewhat more inhibitory than was the same fraction from control livers (Table 2). This effect of diabetes was close to statistical significance, but the magnitude of the effect is trivial (see the Discussion section).

Importance of substrate. The results described above tend to favour the hypothesis that chronic diabetes causes a loss of synthase phosphatase protein. However, assays of synthase phosphatase with ^{32}P -labelled synthase from muscle as substrate impose a modification of this hypothesis. It is clear that the induction of severe chronic diabetes caused an apparent 90% decrease in syn-

these phosphatase activity when measured on hepatic synthase *b*, but a loss of less than 50% when the same liver homogenates were assayed in the de-

Fig. 2. Correlation between the activity of synthase phosphatase and the maximal extent of synthase activation in hepatocytes

The activity of synthase phosphatase was measured on hepatic synthase *b* in a dilute homogenate of a lobe of the liver from which hepatocytes were isolated. The latter were incubated with 60 mM-glucose, and the degree of conversion of synthase *b* into *a* was recorded after incubation for 25–30 min (see Fig. 1a). Δ , Control rats; $\circ$, rats injected with alloxan 40h, 52h, 72h or 90h before the experiment; $\bullet$, moderately 92h-diabetic rats, treated subsequently with insulin for 20h, 44h or 68h. Correlation coefficient $r = 0.87$ ($P < 0.001$).

phosphorylation of labelled synthase *b* from muscle (Fig. 3). It is shown in Fig. 4 that the activation of hepatic synthase *b* and the dephosphorylation of ^{32}P -labelled muscle synthase have a quite different requirement for the S- and G-components of synthase phosphatase. A specific diabetes-induced loss of one component would therefore be a distinct possibility.

Specific loss of G-component. When liver extracts from control animals were fractionated by high-speed centrifugation, little synthase phosphatase activity was recovered, but the activity was largely restored on combination of the high-speed supernatant and the resuspended glycogen + microsomal fraction (Table 3). This fractionation therefore yields a separation between the soluble S-component and the particulate G-component, similar to that obtained by the procedure with starved rats (Doperé *et al.*, 1980; Fig. 4a). Diabetic-rat livers, with decreased activities of synthase phosphatase, yielded subcellular fractions with barely measurable synthase phosphatase (Table 3). Cross-combination between subcellular fractions from diabetic and control animals indicated that the S-component was largely present in the diabetic liver; in contrast, there appears to be a loss of G-component, and the importance of this loss was proportional to the severity of the diabetic state.

Discussion

Nature of the intrahepatic defect in glycogen synthesis

Hepatocytes from chronically diabetic rats respond in a deficient way to a massive glucose load.

Fig. 3. Activation of liver synthase (a) and dephosphorylation of muscle synthase (b) by liver homogenates from control and diabetic rats

Liver homogenates from three control and three severely diabetic rats (plasma glucose = 57 ± 14 mM) were used at a final concentration of 2% (a) or 4% (b). The muscle synthase had been phosphorylated with a synthase kinase independent of either cyclic AMP or Ca^{2+} (kinase F_A). Results similar to those shown in (b) were obtained in parallel experiments with muscle synthase phosphorylated with either cyclic AMP-dependent protein kinase or phosphorylase kinase (results not shown).

Fig. 4. Activation of liver synthase (a) and dephosphorylation of muscle synthase (b) by S- and/or G-component. The components, isolated from normal glucagon-treated rats ($n = 3-5$), were used at a relative concentration of 2%. The preparations of liver and muscle synthase were those used in the experiments shown in Fig. 3.

Table 3. Synthase phosphatase activity in subcellular liver fractions from control and diabetic rats

Supernatants and high-speed pellets (mainly glycogen and microsomal fraction) were prepared from low-speed extracts of control and diabetic livers as described in the Experimental section. Three pooled livers were used for the 'control' fractionation in each experiment. Synthase phosphatase activity was measured with hepatic synthase *b* as substrate. The plasma glucose concentrations were 70 ± 5 mM ($n = 3$) and 38 ± 4 mM ($n = 2$) in the severely and moderately diabetic groups respectively. Within each experiment, the synthase phosphatase activity measured in the recombined supernatant and pellet from the control livers has been taken as 100%.

Cellular fraction	Synthase phosphatase (relative activity)			
	Expt. 1		Expt. 2	
	Control	Severely diabetic	Control	Moderately diabetic
Extract	146	9 ± 4	144	28 ± 5
Supernatant	15	6 ± 2	11	9 ± 4
Pellet	6	4 ± 2	8	3 ± 1
Supernatant + pellet	100	11 ± 2	100	25 ± 5
Supernatant (control) + pellet (diabetic)		14 ± 4		23 ± 1
Supernatant (diabetic) + pellet (control)		80 ± 11		99 ± 20

In severe diabetes, no conversion of synthase *b* into *a* occurs (Bollen *et al.*, 1983). In diabetes of moderate severity, the rate of synthase activation is decreased, as well as the final extent of activation (Fig. 1a). These observations could in principle be explained by either of two hypotheses proposed to explain the impaired activation of glycogen synthase in liver homogenates from diabetic rats: (i) several groups have reported a considerably decreased activity of synthase phosphatase, when measured with exogenous synthase *b* as substrate (Tan & Nuttall, 1976; Miller, 1978; Gold *et al.*, 1979); (ii) it has also been reported that synthase *b* in diabetic liver is resistant to the action of normal synthase phosphatase (Tan & Nuttall, 1976; Akatsuka *et al.*, 1983).

Other observations, however, indicate that the decrease in synthase phosphatase activity is of

major importance in the observed impairment of glycogen synthesis. (i) The diabetes-induced decrease in the rate of synthase activation was similar, whether endogenous or exogenous synthase *b* served as substrate; the decrease was even more marked with exogenous substrate (Fig. 1b and related text). The reverse would have been expected if synthase *b* in the diabetic-rat livers were less susceptible to activation. (ii) As shown in Fig. 2, the extent of synthase activation in isolated cells corresponded well to the measured activity of synthase phosphatase; this relationship was valid during the 4-day period of decrease after the injection of alloxan, as well as during the subsequent insulin-induced restoration over 3 days. (iii) A change in the properties of synthase *b* does not appear to be an obligatory feature of diabetes. At variance with the data of Tan & Nuttall (1976), we

found no evidence for some synthase *b* being in a non-substrate form in diabetic liver (Fig. 1*b*). Also, in contrast with the observations by Akatsuka *et al.* (1983), Bahnak & Gold (1982) reported that synthase *b* in diabetic liver behaved like the normal *b* enzyme with respect to M_r , kinetic properties and susceptibility to activation by phosphatase from control livers. It has been suggested that the alterations involving synthase *b* might be specific for streptozotocin-diabetes, as opposed to alloxan-diabetes (Akatsuka *et al.*, 1983). This possibility requires a direct comparative investigation.

Although it remains questionable whether or not the properties of synthase *b* as substrate contribute to the intrahepatic defect in chronic diabetes, the decrease in synthase phosphatase activity emerges as the decisive event. Our observations on a slower and less complete activation of synthase in hepatocytes from diabetic rats (Bollen *et al.*, 1983; Fig. 1*a*) are therefore best explained by the failure of an impaired synthase phosphatase activity to overcome the activity of synthase kinases. *In vivo*, the situation may conceivably be further aggravated by an increased activity of cyclic AMP-dependent protein kinase, as a result of the hyperglucagonaemia in the diabetic state.

The inactivation of phosphorylase proceeds normally in glucose-stimulated hepatocytes from rats with severe chronic diabetes (Bollen *et al.*, 1983). Therefore we did not expect major changes in the activity of phosphorylase phosphatase. In previous work a mild decrease has been reported; those observations were made on extracts prepared from frozen livers (Khandelwal *et al.*, 1977; Miller *et al.*, 1981) or in a hypo-osmotic medium (Tan & Nuttall, 1976). However, in such conditions there is an important release of partially latent phosphorylase phosphatase from ruptured organelles, as well as proteolytic activation of latent enzyme (Doperé & Stalmans, 1982). We have therefore checked the activity of phosphorylase phosphatase in gel-filtered extracts from fresh livers, homogenized and incubated in conditions of presumed minimal organelle damage. In mild diabetes, no activity change was noted. In contrast, a 3-fold increase was observed with preparations from severely diabetic rats. However, the latter observation does not correlate with the response of intact hepatocytes to glucose (Bollen *et al.*, 1983). It could be possible therefore that the increased phosphorylase phosphatase activity measured *in vitro* is an artifact generated by the homogenization; the cause may be an increased susceptibility of cell organelles to mechanical damage in the liver of the severely diabetic animal.

Nature of the decreased synthase phosphatase activity

Since phosphorylase *a* is a strong inhibitor of

synthase phosphatase, it has been considered as a possible cause of the decreased synthase phosphatase activity in diabetes (Miller *et al.*, 1981). This hypothesis can be eliminated, since the activity of synthase phosphatase remained low when phosphorylase *a* was removed by conversion into the *b* form (Tan & Nuttall, 1976) or by antibodies (Miller *et al.*, 1981), and also when the inhibitory effect of phosphorylase *a* was cancelled by the presence of AMP and Mg^{2+} (throughout the present work). In general, the presence of an inhibitor in diabetic liver seems an unlikely explanation for the decreased synthase phosphatase activity. A transferable inhibitor can be ruled out, since on mixing of homogenates from control and diabetic rats the activity was additive (Miller *et al.*, 1981; Table 2). Khandelwal *et al.* (1977) have reported that a greater amount of a heat-stable inhibitor protein of phosphorylase phosphatase could be extracted from the livers of chronically diabetic rats as compared with control animals and insulin-treated diabetics. In our hands, heated liver extracts from severely diabetic rats were slightly more inhibitory to normal synthase phosphatase than were similar preparations from control rats (Table 2). It is conceivable that the effect of diabetes might have been more significant if it were not superimposed on the large, presumably non-specific, inhibition generated by the heating of a control liver extract. However, the important point is that the extent of the specific diabetes-induced heat-stable inhibition is negligible in comparison with the decrease in synthase phosphatase activity caused by the diabetes.

Although the available evidence with inhibitors of protein synthesis is not entirely conclusive (Haverstick *et al.*, 1979; Miller, 1979), the negative results discussed above tend to favour the hypothesis of a decreased concentration of synthase phosphatase protein in chronic diabetes. However, it is striking that, in all the reports on a very low hepatic activity of synthase phosphatase, the enzyme was assayed by the activation of synthase *b* (Tan & Nuttall, 1976; Miller, 1978; Gold *et al.*, 1979; Miller *et al.*, 1981; the present work). When we assayed the enzyme by the dephosphorylation of ^{32}P -labelled synthase from muscle, the diabetes-induced decrease in the phosphatase activity was much less impressive. The explanation turned out to be a fairly specific decrease in the activity of the G-component of synthase phosphatase. The importance of the decrease was furthermore related to the severity of the diabetic state.

The loss of G-component in diabetes could be caused by a decreased synthesis, which may be under insulin control, but it may also result from an enhanced loss of functional enzyme. In fact, some observations on diabetes could be rationalized

more easily in terms of hyperglycaemia than of lack of insulin. Thus Golden *et al.* (1979) found that the glycogenic response of hepatocytes was considerably improved by starvation of the diabetic rats for 24 h. Synthase phosphatase activity was also doubled in these conditions (Tan & Nuttall, 1976). It is known that haemoglobin and many plasma proteins become non-enzymically glucosylated as a result of hyperglycaemia (Bunn, 1981; Day *et al.*, 1979). Loss of functional G-component by glucosylation is a hypothesis that requires further investigation.

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References

- Akatsuka, A., Singh, T. J. & Huang, K. P. (1983) *Arch. Biochem. Biophys.* **220**, 426–434
- Bahnak, B. R. & Gold, A. H. (1982) *Arch. Biochem. Biophys.* **213**, 492–503
- Bollen, M., Hue, L. & Stalmans, W. (1983) *Biochem. J.* **210**, 783–787
- Bunn, H. F. (1981) *Am. J. Med.* **70**, 325–330
- Dahlqvist, A. (1961) *Biochem. J.* **80**, 547–551
- Day, J. F., Thornburg, R. W., Thorpe, S. R. & Baynes, J. W. (1979) *J. Biol. Chem.* **254**, 9394–9400
- Doperé, F. & Stalmans, W. (1982) *Biochem. Biophys. Res. Commun.* **104**, 443–450
- Doperé, F., Vanstapel, F. & Stalmans, W. (1980) *Eur. J. Biochem.* **104**, 137–146
- Gold, A. H., Dickemper, D. & Haverstick, D. M. (1979) *Mol. Cell. Biochem.* **25**, 47–59
- Golden, S., Wals, P. A., Okajima, F. & Katz, J. (1979) *Biochem. J.* **182**, 727–734
- Haverstick, D. M., Dickemper, D. & Gold, A. H. (1979) *Biochem. Biophys. Res. Commun.* **87**, 177–183
- Hue, L., Bontemps, F. & Hers, H. G. (1975) *Biochem. J.* **152**, 105–114
- Khandelwal, R. L., Zinman, S. M. & Zebrowski, E. J. (1977) *Biochem. J.* **168**, 541–548
- Laskov, R. & Margoliash, E. (1963) *Bull. Res. Council. Isr. Sect. A* **4**, 351–362
- Madsen, N. B., Kasvinsky, P. J. & Fletterick, R. J. (1978) *J. Biol. Chem.* **253**, 9097–9101
- Miller, T. B., Jr. (1978) *Am. J. Physiol.* **234**, E13–E19
- Miller, T. B., Jr. (1979) *Biochim. Biophys. Acta* **583**, 36–46
- Miller, T. B., Jr., Hazen, R. & Lerner, J. (1973) *Biochem. Biophys. Res. Commun.* **53**, 466–474
- Miller, T. B., Jr., Vicalvi, J. J., Jr. & Garnache, A. K. (1981) *Am. J. Physiol.* **240**, E539–E543
- Mvumbi, L., Doperé, F. & Stalmans, W. (1983) *Biochem. J.* **212**, 407–416
- Nimmo, H. G., Proud, C. G. & Cohen, P. (1976) *Eur. J. Biochem.* **68**, 21–30
- Stalmans, W., De Wulf, H. & Hers, H. G. (1971) *Eur. J. Biochem.* **18**, 582–587
- Stalmans, W., De Wulf, H., Hue, L. & Hers, H. G. (1974a) *Eur. J. Biochem.* **41**, 127–134
- Stalmans, W., Laloux, M. & Hers, H. G. (1974b) *Eur. J. Biochem.* **49**, 415–427
- Tan, A. W. H. & Nuttall, F. Q. (1976) *Biochim. Biophys. Acta* **445**, 118–130
- Whitton, P. D. & Hems, D. A. (1975) *Biochem. J.* **150**, 153–165
- Yang, S.-D., Vandenheede, J. R., Goris, J. & Merlevede, W. (1980) *J. Biol. Chem.* **255**, 11759–11767