Glycogen Metabolism of Guinea-Pig Liver in Experimental Bagassosis

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Biochemical investigations have revealed various metabolic disturbances in guinea-pig lungs, associated with experimental bagassosis (Singh, Mitra, Venkitasubramanian & Viswanathan, 1960; Singh, Venkitasubramanian & Viswanathan, 1961). Singh, Venkitasubramanian & Viswanathan (1962) noted changes in the cellular constituents of guinea-pig lungs in experimental bagassosis: these included a remarkable increase in the glycogen content. These findings indicate abnormal carbohydrate metabolism in the lungs of guinea pigs exposed to bagasse dust. In view of the fundamental importance of the liver in carbohydrate metabolism of the body, hepatic glycogen metabolism was investigated.

To evaluate the biochemical response of guinea-pig liver to experimental bagassosis, estimations of cellular constituents of liver such as protein, glycogen and water were carried out. Also, certain enzymes of glycogenolysis and glycolysis, namely α -glucan phosphorylase (α -1 $\rightarrow$ 4-glucan orthophosphate glucosyltransferase, EC 2.4.1.1), glucose 6-phosphatase (D-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9), glucose phosphate isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) and aldolase (ketose 1-phosphate aldehyde-lyase, EC 4.1.2.7), of livers of guinea pigs exposed to bagasse dust were assayed; similar determinations were made on the livers of normal untreated guinea pigs.

MATERIALS AND METHODS

Materials

Chemicals. Glycogen (oyster) was obtained from British Drug Houses Ltd. Glucose 1-phosphate (potassium salt), glucose 6-phosphate (potassium salt), fructose 1,6-diphosphate (barium salt) and tris (Sigma 121) were products of Sigma Chemical Co., St Louis, U.S.A. The barium salt of fructose 1,6-diphosphate was converted into the potassium salt before use.

Animals. Young guinea pigs (300–500 g.) were selected and paired for age, weight and sex. One animal of each pair was exposed to bagasse dust for the prescribed period by the experimental device of Singh *et al.* (1960), and the other served as the corresponding control. Animals were starved for 24 hr. before being killed.

Tissue collection. Guinea pigs were stunned by a blow on the head and exsanguinated by decapitation. No anaesthetic was used as this would have been likely to cause metabolic disturbances (Drucker *et al.* 1959). The liver was

quickly excised and freed from adhering blood with filter paper. Samples of the tissues were cut out, weighed on a torsion balance, dropped into 30% (w/v) KOH (Cori, 1932) and set aside for glycogen estimation. The rest of the tissue was kept in a glass container chilled in ice and used for other estimations.

Preparation of homogenate. Homogenates (10%, w/v) were prepared by grinding weighed samples of tissue in a pre-chilled Potter–Elvehjem homogenizer (with glass tube and Teflon pestle, supplied by Arthur H. Thomas and Co., Philadelphia, U.S.A.) with appropriate ice-cooled solutions. The homogenates were stored at 0° and portions withdrawn for enzymic assays after suitable dilution. Assays were always started soon after preparing the homogenates and the dilutions were carried out immediately before the analysis.

Estimation of cellular constituents

Moisture. Portions of the homogenates were dried to constant weight in an electric oven at 80–100°. When salt solutions were used for the preparation of homogenates, dry weights were corrected for the amount of salt present.

Protein. Portions of homogenates (10%, w/v) were taken for nitrogen estimation by the micro-Kjeldahl method. Protein content was calculated by multiplying the value for nitrogen by the factor 6.25.

Glycogen. The tissue samples were digested by boiling with 30% (w/v) KOH and the glycogen therein was isolated and purified by the method of Good, Kramer & Somogyi (1933). Finally, after dissolving the glycogen precipitate in water and diluting appropriately, portions were analysed by the method of Montgomery (1957).

Enzyme assays

α -Glucan phosphorylase. The method of Shull, Ashmore & Mayer (1956), as modified by Singh *et al.* (1961), was adopted for the assay of this enzyme, with liver homogenate (10%, w/v) in 0.1M-sodium fluoride. The reaction was conducted at 30° for 30 min. The amount of the enzyme which liberates 1 μ mole of orthophosphate/min. at 30° and pH 5.8 is defined as 1 unit of α -glucan phosphorylase.

Glucose 6-phosphatase. The method of Swanson (1950), as modified by Shull *et al.* (1956), was adopted for the assay of glucose 6-phosphatase activity, with liver homogenate (10%, w/v) in chilled glass-distilled water. The reaction was conducted for 30 min. at 30°. The inorganic phosphorus liberated was estimated by the method of Sumner (1944). The amount of enzyme which liberates 1 μ mole of orthophosphate/min. at 30° and pH 6.4 is defined as 1 unit of glucose 6-phosphatase.

Glucose phosphate isomerase. The method of Glock, McLean & Whitehead (1956) was followed for the assay of this enzyme, with 0.2% liver homogenate in 0.15M-KCl

containing KHCO_3 (8 ml. of 0.02M- KHCO_3 /l.), but glycylglycine buffer was replaced by 0.05M-tris buffer, pH 7.6 (Singh *et al.* 1961). The amount of enzyme which produces 1 μ mole of fructose 6-phosphate/min. at 37° and pH 7.6 is defined as 1 unit of glucose phosphate isomerase.

Aldolase. The method of Sibley & Lehninger (1949) was adopted for this assay, with 0.5% liver homogenate in glass-distilled water. The reaction was conducted at 38° for 15 min. The other details were essentially as described by Singh *et al.* (1961). The amount of enzyme that acts on 1 μ mole of fructose 1,6-diphosphate/min. at 38° and pH 8.6 is defined as 1 unit of aldolase.

Experimental procedure

To obtain data of statistical significance, a minimum of seven animals of each of the experimental and the control groups were killed. To minimize variations, the killing of an animal of one group was followed almost immediately

by that of one of the other group, and assays on the tissues of a control and an experimental guinea pig were conducted simultaneously. The various estimations were carried out on the tissue samples of individual animals, and results are expressed as means $\pm$ s.e. The statistical significance of the findings was calculated, $P \leq 0.05$ being considered to be significant.

RESULTS

Table 1 shows the liver weight:body weight ratio and protein and glycogen contents of liver of normal (control) and experimental guinea pigs (exposed to bagasse dust for 430 hr.).

As judged by body weight, growth of guinea pigs exposed to bagasse dust was inhibited. Even though there was no marked enlargement of liver, an appreciable increase in the liver weight:body

Table 1. *Effect of experimental bagassosis on cellular constituents of guinea-pig liver*

Methods of analysis are described in the text. Experimental animals were exposed to bagasse dust for 430 hr. Figures in parentheses indicate the number of experiments performed.

	Normal (7)	Experimental (7)
Water content (%)	72.8	69.8
Liver wt.:body wt. ratio	0.026	0.035
Protein		
Content (g./100 g. dry wt. of liver)	80.2	58.4
Content (g./100 g. of body wt.)	0.57	0.61
Total (g.)	3.53	3.24
Glycogen		
Content (g./100 g. dry wt. of liver)	0.85 $\pm$ 0.18	2.27 $\pm$ 0.2 [$P < 0.001$]
Content (g./100 g. of body wt.)	6.1 $\pm$ 1.0	23.7 $\pm$ 1.5 [$P < 0.001$]
Total (mg.)	37.3 $\pm$ 5.5	124.4 $\pm$ 6.8 [$P < 0.001$]

Table 2. *Effect of experimental bagassosis on α -glucan phosphorylase, glucose 6-phosphatase, glucose phosphate isomerase and aldolase activities of guinea-pig liver*

Details of experimental procedures and enzyme units are described in the Materials and Methods section. Values are given as means $\pm$ s.e. (with number of experiments shown in parentheses).

Period of exposure to bagasse dust (hr.)	Enzyme activities		
	Units/g. wet wt. of liver	Units/g. dry wt. of liver	Units/100 g. of body wt.
α-Glucan phosphorylase (7)			
Nil	15.2 $\pm$ 1.4	54.5 $\pm$ 5.1	39.7 $\pm$ 3.0
230-270	12.2 $\pm$ 3.3 [$P > 0.4$]	42.6 $\pm$ 11.7 [$P > 0.4$]	37.1 $\pm$ 8.2 [$P > 0.7$]
Glucose 6-phosphatase (7)			
Nil	7.0 $\pm$ 0.7	25.1 $\pm$ 3.2	18.3 $\pm$ 2.0
230-270	6.7 $\pm$ 0.9 [$P > 0.5$]	23.3 $\pm$ 3.0 [$P > 0.2$]	20.4 $\pm$ 2.3 [$P > 0.25$]
Glucose phosphate isomerase (8)			
Nil	102.6 $\pm$ 4.7	379.9 $\pm$ 17.4	276.0 $\pm$ 12.6
230-270	172.0 $\pm$ 23.1 [$P < 0.005$]	573.4 $\pm$ 76.3 [$P < 0.005$]	520.7 $\pm$ 69.0 [$P < 0.005$]
Aldolase (8)			
Nil	3.29 $\pm$ 0.16	12.1 $\pm$ 0.6	8.8 $\pm$ 0.4
230-270	4.04 $\pm$ 0.2 [$P < 0.025$]	13.5 $\pm$ 0.7 [$P < 0.01$]	12.2 $\pm$ 0.6 [$P < 0.001$]

weight ratio of these experimental guinea pigs was noted (Table 1). There was no significant alteration in the water content of the liver of the exposed animals.

Glycogen analysis on the liver samples of seven normal and seven experimental animals exposed to bagasse dust for 430 hr. (Table 1) revealed a marked increase in the glycogen content of liver of the latter, especially when the results were expressed on the basis of total glycogen of liver/100 g. of body weight. On this basis the increase was almost 300 %.

Six normal and six experimental animals (exposed to bagasse dust for 430 hr.) were killed and the nitrogen in the samples of pooled liver was estimated by the micro-Kjeldahl method. A noticeable decrease (nearly 30 %) in the protein content (expressed as percentage in dry tissue) of liver of the guinea-pigs exposed to bagasse dust was evident (Table 1). However, since the liver weight:body weight ratio was increased after exposure to bagasse dust, the total protein content of liver/100 g. of body weight was almost unaffected.

Assays of the glycogenolytic enzymes α -glucan phosphorylase and glucose 6-phosphatase, carried out on the liver samples of seven normal and seven experimental animals, indicate that the activities of these enzymes were unaffected by exposure to bagasse dust for 230–270 hr. (Table 2).

An appreciable enhancement in the activities of the glycolytic enzymes glucose phosphate isomerase and aldolase is evident from assays on the liver samples of eight normal and eight experimental guinea pigs, exposed to bagasse dust for 230–270 hr. (Table 2). Results, expressed on the basis of units/100 g. of body weight, show increases of 100 and 50 % in the activities of glucose phosphate isomerase and aldolase respectively.

DISCUSSION

Since all the studies were not performed on the same group of animals, it is likely that conversion of values from one basis of expressing the results to another may give different ratios for different assays. The manner in which the experimental findings are set out is of considerable importance in the interpretation of data, especially for a comparative study of normal and pathological cases. In the present discussion emphasis is laid mainly on the total available activity, i.e. total activity of the liver corresponding to 100 g. of body weight.

A marked increase in the glycogen content of guinea-pig liver results from exposure to bagasse dust (Table 1). Excessive accumulation of glycogen in one or more organs of the body is a conspicuous abnormality in several diseases, notably in 'glycogen-storage disease'.

The glycogen content of hepatic cells depends on the efficiency of glycogenolysis, glycogenesis and gluconeogenesis, operating simultaneously. A depression of the degradative process or an augmentation of the synthetic pathway of glycogen metabolism would eventually result in glycogen infiltration. In some examples of glycogen-storage disease, the accumulation of glycogen has been attributed to the inhibition of glycogen breakdown as a result of abnormal glycogen structure. In other cases various enzymic lesions have been reported to occur at the levels of α -glucan phosphorylase, glucose 6-phosphatase and amylo-1 $\rightarrow$ 6-glucosidase (Stetten & Stetten, 1960). In irradiated animals excessive glycogen deposition in liver has been ascribed to enhanced glycogenesis (Nerurkar & Sahasrabudhe, 1959) and gluconeogenesis (McKee, 1952; Morehouse & Searcy, 1955; McKee & Brin, 1956) and to reduced glycogenolysis (Ross & Ely, 1951; Denson *et al.* 1954).

In the present investigation the α -glucan-phosphorylase activity of guinea-pig liver was unaffected by exposure to bagasse dust (Table 2). In view of the current belief that α -glucan phosphorylase is involved exclusively in the process of glycogen breakdown (Robbins & Lipmann, 1959; Leloir & Goldemberg, 1960; Villar-Palasi & Lerner, 1960; Brekenridge & Crawford, 1960), the present findings (Table 2) may indicate that the capacity of guinea-pig liver to degrade normal glycogen to glucose 1-phosphate is not affected in experimental bagassosis.

The results with glucose 6-phosphatase obtained in the present investigation (Table 2) show that the capacity of guinea-pig liver to dephosphorylate glucose 6-phosphate to glucose is not affected by the exposure to bagasse dust. It may be concluded, therefore, that the biochemical lesion responsible for hepatic glycogen infiltration in experimental bagassosis is not the α -glucan-phosphorylase or glucose 6-phosphatase reactions.

In the present case where glycogen breakdown does not seem to be depressed, excessive accumulation of glycogen in liver might be expected to result from an increase in glycogen synthesis (either glycogenesis or gluconeogenesis). A study of the uridine diphosphate glucose-linked pathway of glycogen synthesis in the present investigation could have proved to be of immense help in elucidating the mechanism of hepatic glycogen infiltration in experimental bagassosis. But, owing to lack of proper facilities, this study could not be undertaken in the present work. Gluconeogenesis involves a reversal of various glycolytic reactions. Hence, where there is an augmented gluconeogenesis, an increase in the activities of the reversible enzymes of the glycolytic pathway is to be expected. We have observed a marked increase in the

activities of glucose phosphate isomerase and aldolase in livers of guinea pigs exposed to bagasse dust, and this may indicate increased gluconeogenesis.

SUMMARY

1. Chemical analysis of the cellular constituents of guinea-pig liver revealed that, though there was a marked increase in the glycogen content and an appreciable decrease in the percentage of protein in dry tissue, no alteration in the total protein/100 g. of body weight and water content of the tissue as a result of exposure to bagasse dust could be noted.

2. Activities of α -glucan phosphorylase, glucose 6-phosphatase, glucose phosphate isomerase and aldolase in livers of normal guinea pigs and of guinea pigs exposed to bagasse dust were assayed. Though no significant change in the activities of the first two enzymes could be observed, a marked increase in that of the latter two enzymes as a result of exposure was apparent.

3. Excessive glycogen accumulation in the liver of guinea pigs exposed to bagasse dust is discussed in the light of the enzymic findings.

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REFERENCES

- Breckenridge, B. M. & Crawford, E. J. (1960). *J. biol. Chem.* **235**, 3054.
- Cori, G. T. (1932). *J. biol. Chem.* **96**, 259.
- Denson, J. R., Gray, E. J., Gray, J. L., Herbert, F. J., Tew, J. T. & Jensen, S. (1954). *Proc. Soc. exp. Biol., N.Y.*, **82**, 707.
- Drucker, W. R., Costley, C., Stults, R., Holden, W. D., Craig, J., Miller, M., Hofmann, N. & Woodward, H. (1959). *Metabolism*, **8**, 827.
- Glock, G. E., McLean, P. & Whitehead, J. K. (1956). *Biochem. J.* **63**, 520.
- Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
- Leloir, L. F. & Goldemberg, S. H. (1960). *J. biol. Chem.* **235**, 919.
- McKee, R. W. (1952). *Fed. Proc.* **11**, 256.
- McKee, R. W. & Brin, M. (1956). *Arch. Biochem. Biophys.* **61**, 390.
- Montgomery, R. (1957). *Arch. Biochem. Biophys.* **67**, 378.
- Morehouse, M. G. & Searcy, R. L. (1955). *Science*, **122**, 158.
- Nerurkar, M. K. & Sahasrabudhe, M. B. (1959). *Brit. J. exp. Pathol.* **40**, 318.
- Robbins, P. W. & Lipmann, F. (1959). In *Ciba Foundation Symp., The Regulation of Cell Metabolism*, p. 188.
- Ross, M. H. & Ely, J. O. (1951). *J. cell. comp. Physiol.* **37**, 163.
- Shull, K. H., Ashmore, J. & Mayer, J. (1956). *Arch. Biochem. Biophys.* **62**, 210.
- Sibley, J. A. & Lehninger, A. L. (1949). *J. biol. Chem.* **177**, 859.
- Singh, V. N., Mitra, S., Venkatasubramanian, T. A. & Viswanathan, R. (1960). *Enzymologia*, **21**, 345.
- Singh, V. N., Venkatasubramanian, T. A. & Viswanathan, R. (1961). *Biochem. J.* **78**, 728.
- Singh, V. N., Venkatasubramanian, T. A. & Viswanathan, R. (1962). *Brit. J. exp. Pathol.* (in the Press).
- Stetten, D., jun. & Stetten, M. R. (1960). *Physiol. Rev.* **40**, 505.
- Sumner, J. B. (1944). *Science*, **100**, 413.
- Swanson, M. A. (1950). *J. biol. Chem.* **184**, 647.
- Villar-Palasi, C. & Lerner, J. (1960). *Arch. Biochem. Biophys.* **86**, 270.

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The Photometabolism of Propionate by *Rhodospirillum rubrum*

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The studies of Elsdon & Ormerod (1956) on the effect of monofluoroacetate on the photometabolism of propionate and succinate by *Rhodospirillum rubrum* suggested that these compounds were metabolized by a common pathway. Subsequently, Ormerod (1956) found that the incorporation of ^{14}C into *R. rubrum* during the photometabolism of propionate in the presence of $^{14}\text{CO}_2$ was three or

four times as great as the net fixation of carbon dioxide measured manometrically, whereas when butyrate was photometabolized in the presence of $^{14}\text{CO}_2$ the net fixation of carbon dioxide measured manometrically was equal to the amount of ^{14}C incorporated. These results suggested that carbon dioxide plays a special role in the photometabolism of propionate. The role of carbon dioxide was further emphasized by the work of Clayton and his colleagues (Clayton, Dettmer & Robertson, 1957; Clayton, 1957), who showed that the metabolic

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