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Pathways of Glucose Catabolism in Rat Liver in Alloxan Diabetes and Hyperthyroidism

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A previous publication (Glock & McLean, 1955) reported striking alterations in levels of activity of enzymes of the hexose monophosphate oxidative pathway of glucose metabolism both in alloxan diabetes and hyperthyroidism. It was found that levels of activity of both glucose 6-phosphate and 6-phosphogluconate dehydrogenases were markedly reduced in the livers of diabetic rats and greatly increased after thyroxine treatment. In an attempt to substantiate these findings and to assess the relative importance of glycolytic and non-glycolytic pathways of carbohydrate metabolism, the utilization of [1-^{14}C]glucose and [6-^{14}C]glucose by liver slices from normal, diabetic and hyperthyroid rats has been determined by measuring the conversion of these labelled substrates into $^{14}CO_2$. In addition to these isotope measurements, the levels of activity of glucose 6-phosphatase and phosphoglucose isomerase have also been determined, since the former is one of the factors regulating the availability of glucose 6-phosphate, the common substrate for both metabolic pathways, whilst striking alterations in activity of phosphoglucose isomerase might be expected to influence the proportion of glucose 6-phosphate metabolized via the glycolytic route.

EXPERIMENTAL

Materials

D-*Glucose 6-phosphate* (*G 6-P*). This was a preparation of the heptahydrate of the barium salt obtained from Sigma Chemical Co.

6-*Phosphogluconate* (*6-PG*). This was a preparation of the barium salt used previously (Glock & McLean, 1953).

[1-^{14}C]*Glucose and* [6-^{14}C]*glucose*. [1-^{14}C]Glucose was obtained from the Radiochemical Centre, Amersham, and [6-^{14}C]glucose from Dr H. S. Isbell, National Bureau of Standards, Washington.

Methods

Estimation of G 6-P dehydrogenase and 6-PG dehydrogenase activities. These were determined spectrophotometrically by following the rate of reduction of triphosphopyridine nucleotide (TPN) at 340 mμ. in 1 cm. cells in a Hilger Uvispek spectrophotometer as described previously (Glock & McLean, 1953). In the assay of G 6-P dehydrogenase activity, the reaction mixture consisted of 0·5 ml. of 0·25 M glycylglycine (pH 7·6), 0·5 ml. of 0·1 M-$MgCl_2$, 0·1 ml. of 6-PG dehydrogenase prepared from rat liver by fractionation with $(NH_4)_2SO_4$, followed by dialysis (Glock & McLean, 1953), 0·1 ml. of liver supernatant and 0·2 mg. of TPN in a total volume of 2·4 ml. The reaction was started by the addition of 0·1 ml. of 0·05 M G 6-P to both cells, the blank being devoid of TPN. In the assay of 6-PG dehydrogenase activity, the $(NH_4)_2SO_4$ liver fraction was omitted, G 6-P was replaced by 0·1 ml. of 0·05 M 6-PG and the reaction was carried out in glycylglycine buffer at both pH 7·6 and pH 9·0. A unit of enzyme activity is defined as the quantity of enzyme which reduces 0·01 μmole of TPN/min. at 20°.

Estimation of glucose 6-phosphatase activity. This was determined according to Duve, Pressman, Gianetto, Wattiaux & Applemans (1955). The incubation mixture contained 0·04 M G 6-P, 0·007 M histidine, 0·001 M ethylenediaminetetraacetate (pH 6·5) and 0·2 ml. of 10% (w/v) liver homogenate in a total volume of 1 ml. After incubation for 10 min. at 37°, the reaction was stopped by the addition of an equal volume of 10% trichoroacetic acid, and inorganic P was determined in the filtrate by the method of Fiske & Subbarow (1925). A unit of enzyme activity is defined as the quantity of enzyme which hydrolyses 1 μmole of G 6-P/min. at 37°.

Estimation of phosphoglucose isomerase activity. This assay was based on that described by Slein (1955), for muscle phosphoglucose isomerase, fructose 6-phosphate formation being determined colorimetrically by the method of Roe (1934), which gives approximately 65% of the colour of free fructose. The incubation mixture contained 4 μmoles of G 6-P, 20 μmoles of glycylglycine (pH 7·6), and 0·1 ml. of 0·1% liver homogenate in a total volume of 0·5 ml. The homogenate was prepared in ice-cold isotonic KCl (0·15 M)

containing $KHCO_3$ (8 ml. of 0·02M $KHCO_3$/l.). After incubating for 10 min. at 37°, the reaction was stopped by the addition of 3·5 ml. of 8·3M-HCl, 1 ml. of 0·1% resorcinol in 95% ethanol was added and the mixture heated for 10 min. at 80°. The tubes were cooled and the colours read in 1 cm. cells at 490 mμ. in a Hilger Uvispek spectrophotometer. Under these conditions of assay, where the fructose 6-phosphate formation is less than 15% of that formed at equilibrium and there is no detectable hydrolysis of either G 6-P or fructose 6-phosphate, the measured activities were directly proportional to both enzyme concentration and time. A unit of enzyme activity is defined as the quantity of enzyme which produces 1 μmole of fructose 6-phosphate/min. at 37°.

Determination of deoxyribonucleic acid (DNA). Liver DNA was determined as described previously (Glock & McLean, 1955).

Treatment of animals. The dietary procedure and production of alloxan diabetes and hyperthyroidism were the same as those described previously (Glock & McLean, 1955), except that the diabetic rats were killed 3–4 weeks after injection of alloxan. In the insulin-reversal experiments, the rats were injected thrice, subcutaneously, at 12 hr. intervals with 10 units of insulin followed by 20 units intraperitoneally 1 hr. before killing. Blood-sugar values were determined periodically. We are indebted to Dr K. Hallas-Møller for a gift of crystalline insulin free from hyperglycaemic factor, which was used in all the reversal experiments.

Isotope experiments. Rat-liver slices, approximately 0·4 mm. in thickness, were prepared with a Stadie–Riggs cutter (Stadie & Riggs, 1944). The slices (500 mg.) were put into a special flask (see Chernick, Masoro & Chaikoff, 1950) containing 4·5 ml. of Krebs–Ringer bicarbonate solution [previously gassed with O_2+CO_2 (95:5)] and 0·2 ml. of glucose solution containing approximately 0·2 μc of ^{14}C (in the form of [1-^{14}C]glucose or [6-^{14}C]glucose) in 20 mg. of glucose. The side arm contained 0·5 ml. of 5N-H_2SO_4. After incubation for 90 min. at 38° with O_2+CO_2 (95:5) as the gas phase, 1 ml. of 5N-KOH was introduced into the centre well by injection through the rubber teat and the H_2SO_4 was tipped in from the side arm. A further 60 min. was allowed before the contents of the centre well were transferred and diluted with water to 10 ml. Measured samples of this solution were used for determining $^{14}CO_2$ by gas counting as described by Salmony & Whitehead (1954).

RESULTS

Alloxan diabetes

The results for the percentage conversion of [1-^{14}C]-glucose and [6-^{14}C]glucose into $^{14}CO_2$ by liver slices from control, diabetic and insulin-treated diabetic rats are given in Table 1. Although the yield of $^{14}CO_2$ from [1-^{14}C]glucose is slightly reduced in the diabetic rats, this decrease is not statistically significant. The yield of $^{14}CO_2$ from [6-^{14}C]glucose, however, is greatly reduced, resulting in a significant increase in the quotient (yield of $^{14}CO_2$ from [1-^{14}C]-glucose)/(yield of $^{14}CO_2$ from [6-^{14}C]glucose), henceforward termed '1-C/6-C quotient' in the diabetic-rat liver. Insulin treatment produces a significant increase above the diabetic level in the yield of $^{14}CO_2$ from both [1-^{14}C]glucose and [6-^{14}C]glucose, and a return of the 1-C/6-C quotient towards the control level. In addition, insulin treatment increases significantly the yield of $^{14}CO_2$ from [1-^{14}C]-glucose above the control level.

The levels of activity of G 6-P and 6-PG dehydrogenases and of phosphoglucose isomerase in the livers of the same control, diabetic and insulin-treated diabetic rats, as were used for the isotope measurements, are given in Table 2. Although, when expressed in units/g. of liver, the level of activity of 6-PG dehydrogenase, but not of G 6-P dehydrogenase, was decreased in the livers of the diabetic rats, there was a significant fall in the activity of both dehydrogenases when expressed in units/mg. DNAP. The failure, in contrast to earlier findings (Glock & McLean, 1955), to obtain a reduction in G 6-P dehydrogenase activity except when expressed in units/mg. DNAP can largely be attributed to the fact that in the present investigation a more prolonged diabetes resulted in a very marked reduction in liver weight. Insulin treatment of the diabetic rats produced a significant increase above the diabetic level in the liver activities of both G 6-P and 6-PG dehydrogenases, when expressed either in units/g. of liver or in units/mg. DNAP. Insulin also increased the activity of G 6-P dehydrogenase above the control level.

Phosphoglucose-isomerase activity was not altered significantly when expressed in units/g. of liver either in the diabetic or insulin-treated diabetic rats. When expressed in units/mg. DNAP, however, the isomerase activity was significantly reduced in the diabetic rats and significantly increased above the diabetic level as a result of

Table 1. *Yields of* $^{14}CO_2$ *from* [1-^{14}C]*glucose and* [6-^{14}C]*glucose by liver slices from alloxan-diabetic rats and insulin-treated diabetic rats*

Results expressed as means ± S.E.M. For details, see Methods section.

	Control group	Alloxan-diabetic group		*P* Control *v.* diabetic	*P* Diabetic *v.* diabetic + insulin
		No insulin	Insulin		
No. of animals	13	12	10	—	—
Conversion (%) of [1-^{14}C]glucose into $^{14}CO_2$	1·00 ± 0·11	0·76 ± 0·06	1·49 ± 0·28	0·072	0·012
Conversion (%) of [6-^{14}C]glucose into $^{14}CO_2$	0·63 ± 0·04	0·23 ± 0·02	0·66 ± 0·08	<0·001	<0·001
Yield of $^{14}CO_2$ from [1-^{14}C]glucose / Yield of $^{14}CO_2$ from [6-^{14}C]glucose	1·60 ± 0·15	3·72 ± 0·38	2·26 ± 0·25	<0·001	0·007

insulin treatment. Glucose 6-phosphatase activity was not determined, since it has already been shown to be significantly reduced in the livers of diabetic rats (Ashmore, Hastings & Nesbett, 1954; Langdon & Weakley, 1955).

Hyperthyroidism

The results of the isotope experiments with liver slices from control and thyroxine-treated rats are given in Table 3. Although there is no significant difference between the yield of $^{14}CO_2$ from [1-^{14}C]-glucose by liver slices of control and hyperthyroid animals, the yield of $^{14}CO_2$ from [6-^{14}C]glucose is significantly increased as a result of thyroxine treatment. This produces a significant decrease in the 1-C/6-C quotient.

Additional measurements on the livers of the same experimental animals included the determination of levels of activity of G 6-P and 6-PG dehydrogenases, phosphoglucose isomerase and glucose 6-phosphatase. These results are incorporated in Table 4. The marked increases in the levels of activity of both G 6-P and 6-PG dehydrogenases in the livers of the hyperthyroid rats confirm earlier findings (Glock & McLean, 1955), although the isotope results are interpreted as indicating that the increased glucose catabolism is due to greater participation of the glycolytic route. There is also a significant increase in the glucose 6-phosphatase activity of the livers of the hyperthyroid rats. Phosphoglucose isomerase activity was not significantly altered.

Table 2. *Levels of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphoglucose isomerase in the livers of control, alloxan-diabetic and insulin-treated diabetic rats*

Results expressed as means ± S.E.M. For details see Methods section. DNAP, deoxyribonucleic acid phosphorus.

	Control group	Alloxan-diabetic group		*P* Control *v.* diabetic	*P* Diabetic *v.* diabetic + insulin
		No insulin	Insulin		
No. of animals	12	10	10	—	—
Initial body wt. (g.)	224±1	225±2	225±1	—	—
Final body wt. (g.)	274±8	174±8	186±12	—	—
Liver wt. (g.)	10·70±0·39	7·70±0·33	10·09±0·66	—	—
Average daily sugar excretion (g.)	—	7·8±1·2	3·9±0·7	—	—
Blood sugar level (mg./100 ml.)	—	349±39	118±19	—	—
DNAP (μg./g. of liver)	204±8	272±16	206±10	—	—
Glucose 6-phosphate dehydrogenase, pH 7·6					
Units of enzyme/g. of liver	47±3	51±3	71±7	0·150	0·043
Units of enzyme/mg. of DNAP	230±16	187±10	345±28	0·042	<0·001
6-Phosphogluconate dehydrogenase, pH 9·0					
Units of enzyme/g. of liver	193±11	115±5	152±11	<0·001	0·011
Units of enzyme/mg. of DNAP	945±67	423±31	738±68	<0·001	<0·001
6-Phosphogluconate dehydrogenase, pH 7·6					
Units of enzyme/g. of liver	92±7	51±2	64±4	<0·001	0·005
Units of enzyme/mg. of DNAP	450±35	187±12	310±20	<0·001	<0·001
Phosphoglucose isomerase*					
Units of enzyme/g. of liver	88±4	78±4	97±8	0·117	0·072
Units of enzyme/mg. of DNAP	431±37	287±12	471±43	0·008	0·004

* Six rats only in each of these three groups.

Table 3. *Yields of $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose by liver slices from control and thyroxine-treated rats*

Results expressed as means ± S.E.M. For details, see Methods section.

	Control group	Thyroxine-treated group	*P*
No. of animals	8	8	—
Conversion (%) of [1-^{14}C]glucose into $^{14}CO_2$	1·49±0·13	1·68±0·17	0·400
Conversion (%) of [6-^{14}C]glucose into $^{14}CO_2$	0·75±0·06	1·26±0·12	0·003
$\frac{\text{Yield of } ^{14}CO_2 \text{ from [1-}^{14}\text{C]glucose}}{\text{Yield of } ^{14}CO_2 \text{ from [6-}^{14}\text{C]glucose}}$	2·00±0·13	1·34±0·07	<0·001

Table 4. *Levels of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glucose 6-phosphatase and phosphoglucose isomerase in the livers of control and thyroxine-treated rats*

Results expressed as means ± S.E.M. For details, see Methods section.

	Control group	Thyroxine-treated group	*P*
No. of animals	6	6	—
Initial body wt. (g.)	183±2	183±3	—
Final body wt. (g.)	197±3	184±8	—
Liver wt. (g.)	8·77±0·39	7·58±0·49	—
Glucose 6-phosphate dehydrogenase, pH 7·6			
Units of enzyme/g. of liver	64±6	100±10	0·009
Total units of enzyme in liver	562±48	758±68	0·017
6-Phosphogluconate dehydrogenase, pH 9·0			
Units of enzyme/g. of liver	198±10	422±26	<0·001
Total units of enzyme in liver	1736±91	3200±208	<0·001
6-Phosphogluconate dehydrogenase, pH 7·6			
Units of enzyme/g. of liver	69±8	154±13	<0·001
Total units of enzyme in liver	605±72	1168±104	0·001
Glucose 6-phosphatase			
Units of enzyme/g. of liver	18·3±1·5	27·1±1·1	<0·001
Total units of enzyme in liver	158±8	205±13	0·013
Phosphoglucose isomerase			
Units of enzyme/g. of liver	126±7	138±7	0·223
Total units of enzyme in liver	1101±70	1051±103	0·698

DISCUSSION

The use of [1-^{14}C]glucose and [6-^{14}C]glucose for the quantitative assessment of the relative importance of alternative pathways of glucose metabolism has recently been fully discussed by Wood (1955). Although preferential utilization of [1-^{14}C]glucose indicates the participation of a non-glycolytic route, exact quantitative interpretation of the results is hazardous since the calculations involve many assumptions and uncertainties. For this reason no quantitative assessment has been attempted in the present study, which was undertaken with the hope of substantiating, in terms of CO_2 production from the tissue slice, the previous findings (Glock & McLean, 1955) that levels of activity of enzymes of the hexose monophosphate oxidative pathway were markedly reduced in the livers of diabetic rats and strikingly increased as a result of thyroxine treatment.

The present results show that in liver slices from diabetic rats, whereas the formation of $^{14}CO_2$ from [1-^{14}C]glucose is only slightly lower than the control value, that from [6-^{14}C]glucose is considerably reduced. This results in a significant increase in the 1-C/6-C quotient from 1·60 to 3·72, indicating a decrease in the proportion of the total glucose catabolized via glycolysis. The results for $^{14}CO_2$ production from [1-^{14}C]glucose substantiate those for G 6-P dehydrogenase activity, which is not significantly reduced when expressed in units/g. of liver, although there is a marked reduction in units/mg. DNAP. Treatment of the diabetic animals with insulin produced a significant increase in the activity of G 6-P and 6-PG dehydrogenases, both when expressed in units/g. of liver and in units/mg. DNAP. This was accompanied by a significant increase in the yield of $^{14}CO_2$ from [1-^{14}C]glucose, above both diabetic and control levels. The yield of $^{14}CO_2$ from [6-^{14}C]glucose was increased even more by insulin treatment, resulting in a return of the 1-C/6-C quotient towards the control value.

The results obtained with isotopes for liver slices from diabetic rats do not agree with those of Bloom (1955), who found that although the formation of $^{14}CO_2$ from both [1-^{14}C]glucose and [6-^{14}C]glucose was greatly reduced, a lowered value for the 1-C/6-C quotient indicated that a greater proportion of glucose was being catabolized via glycolysis. These discrepancies may perhaps be attributed to the fact that in the experiments of Bloom the diabetes was more severe and of longer duration.

In the case of liver slices from thyroxine-treated rats, the isotope results indicate that the increased glucose catabolism is due to a greater participation of the glycolytic pathway, since the yield of $^{14}CO_2$ from [6-^{14}C]glucose only is increased, and there is a significant fall in the 1-C/6-C quotient from 2·00 in the control group to 1·34 in the hyperthyroid group. It thus appears that in spite of increased activity of enzymes of the hexose monophosphate oxidative pathway in the hyperthyroid-rat liver, the glycolytic pathway competes more effectively for available substrate, the concentration of which is limited by increased glucose 6-phosphatase activity.

SUMMARY

1. Rat-liver slices have been incubated with [1-^{14}C]glucose and with [6-^{14}C]glucose, and the yields of $^{14}CO_2$ from these two substrates have been compared in normal, alloxan-diabetic, insulin-treated diabetic and thyroxine-treated animals. Livers from the same animals have been used for determining the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and of phosphoglucose isomerase. In addition, glucose 6-phosphatase activity has been determined in the control and hyperthyroid-rat livers.

2. In diabetes the formation of $^{14}CO_2$ from [6-^{14}C]glucose is significantly reduced, whereas that from [1-^{14}C]glucose is only very slightly reduced. This indicates a decrease in the relative proportion of glucose catabolized via glycolysis.

3. Treatment of diabetic rats with insulin partially reverses the changes mentioned above by increasing the $^{14}CO_2$ production from both [1-^{14}C]-glucose and [6-^{14}C]glucose, with an accompanying fall in the quotient (yield of $^{14}CO_2$ from [1-^{14}C]-glucose)/(yield of $^{14}CO_2$ from [6-^{14}C]glucose). The levels of activity of glucose 6-phosphate and 6-phosphogluconate dehydrogenases are also increased, glucose 6-phosphate dehydrogenase activity being significantly higher than that of normal liver.

4. After thyroxine treatment $^{14}CO_2$ production from [6-^{14}C]glucose is significantly increased, whereas that from [1-^{14}C]glucose is only very slightly increased, indicating greater participation of the glycolytic pathway.

5. Glucose 6-phosphatase activity is significantly higher in the livers of thyroxine-treated rats.

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The Effects of Salicylate on Oxygen Consumption and Carbohydrate Metabolism in the Isolated Rat Diaphragm

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Salicylate produces a number of effects on carbohydrate metabolism in the normal rat, such as depletion of liver and muscle glycogen (Lutwak-Mann, 1942; Winters & Morrill, 1955), an initial hyperglycaemia (Smith, 1955*a*) and an inhibition of the actions of adrenal corticosteroids in producing glycosuria and hyperglycaemia (Smith, 1952). The depletion of glycogen and antagonism of the effects of adrenal cortical extracts have also been observed with rat-liver slices (Smith, 1955*b*). Salicylate decreases the glycosuria and hyperglycaemia in the diabetic rat (Ingle, 1950; Smith, Meade & Bornstein, 1952) and produces hypoglycaemia in adrenalectomized and hypophysectomized animals (Smith, 1955*a*; Comulada, Carlo & Smith, 1953).

An increased consumption of oxygen after the administration of salicylate occurs in man (Cochran, 1952) and the rat (Meade, 1954), and in isolated tissues such as mouse-liver slices (Sproull, 1954) and rat-brain preparations (Fishgold, Field & Hall, 1951) incubated with salicylate. The possible relation between this increased oxygen consumption and the effects of salicylate on carbohydrate metabolism is of some interest, particularly because of the suggestion (Meade, 1954) that the action of salicylate may resemble that of the dinitrophenols, which interfere with oxidative phosphorylation processes (Loomis & Lipmann, 1948). The present work is concerned with a study of the effects of salicylate on the isolated rat diaphragm, which