ALLOXAN INDUCED CHANGE FROM CARBOHYDRATE TO LIPID OXIDATION IN RATS DETERMINED BY THE PREVALENCE OF CARBON-13 IN EXPIRED CARBON DIOXIDE

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SUMMARY

Carbon dioxide from untreated and alloxanized rats was collected free of atmospheric CO_2 and then analyzed for its stable carbon isotope ($^{13}C/^{12}C$) ratio. Alloxan induced diabetic rats expired CO_2 with a $^{13}C/^{12}C$ ratio less than that for CO_2 collected from untreated rats. The difference in $^{13}C/^{12}C$ ratios indicated that alloxanized rats oxidized more lipid than control rats since rat lipid has a $^{13}C/^{12}C$ ratio less than that of glycogen.

Of the many disturbances correlated with diabetes mellitus one is an increase in fatty acid oxidation (1). Furthermore, it has been stated or implied by various individuals that the onset of diabetes is primarily a result of an alteration in the metabolism of lipids (1,2). An aid to understanding this problem especially in humans, would be a reliable method for determining the relative contribution of lipid or carbohydrate to the respiratory mainstream without having to feed radioactively labeled compounds to the organism. A method which permits determination of the relative contribution of endogenous substrates using the naturally occurring differences in the relative amounts of stable carbon isotopes in metabolites has been developed for plant tissues (3,4).

The rationale for the method using stable carbon isotopes is based upon the fact that the relative content of carbon-12 and carbon-13 in lipids is quite different from that found in other naturally occurring organic compounds (3). The diversity in carbon isotope content can be attributed to the different reactivities exhibited by ^{12}C and ^{13}C during biosynthesis of the

various metabolites generally attributed to the difference in mass of the two stable isotopes. Hence, different classes of metabolites have characteristic $^{13}C/^{12}C$ ratios. In general the percentage of ^{13}C in carbohydrate is greater than that in lipid (5). Furthermore, oxidation of a metabolite results in a $^{13}C/^{12}C$ ratio of respired CO_2 which reflects the $^{13}C/^{12}C$ ratio of the metabolite being oxidized (3). The relative contribution of lipid or carbohydrate plus protein to the total *in vivo* metabolism of tissue can be determined by first collecting respired CO_2 and then comparing its $^{13}C/^{12}C$ ratio with the $^{13}C/^{12}C$ ratios characterizing the potential respiratory substrates (3). This method does not require the feeding of isotopically labeled compounds to an organism since the various endogenous respiratory substrates naturally have different $^{13}C/^{12}C$ ratios.

In this paper we report an investigation which shows that the $^{13}C/^{12}C$ ratio of rat lipid is less than that of carbohydrate and that the $^{13}C/^{12}C$ ratio of expired CO_2 from alloxanized rats is indicative of lipid metabolism and is different from the $^{13}C/^{12}C$ ratio of CO_2 collected from untreated rats.

MATERIALS AND METHODS

Male Wistar rats were starved overnight and then given an injection of alloxan (40 mg per 100 g body weight) two days before use and during this time were given food *ad libitum*. The rats were given 1.5 units of short term insulin two hours before the alloxan injection. The sugar content of the blood and urine was monitored and indicated alloxan diabetes. Fifty percent of the rats died shortly after alloxan treatment.

Rats were placed in one liter glass chamber for CO_2 collection. Ample CO_2 was collected from the rats in less than 8 min., as previously described (3).

Rat liver was homogenized, filtered through cheese cloth and centrifuged at 500 *g*. The supernatant was then centrifuged at 20,000 *g*, and the resultant pellet was suspended in water, washed with n-butanol in a separatory funnel and then centrifuged at 20,000 *g*. The pellet was resuspended in water, washed in

n-butanol and centrifuged. This last purification step was repeated three times. The resultant glycogen pellet was dried at room temperature. Lipid was isolated by the following procedure: The liver residue remaining on the cheese cloth filter, the first low-speed pellet, and the high-speed supernatant were all combined and refluxed for one hour with two volumes of chloroform-methanol (2:1). The organic solvent layer was saved and the bulk of the solvent removed by rotary evaporation. The last bit of solvent was evaporated at low temperature leaving the liver lipid fraction. Both glycogen and lipid samples were combusted at 800°C and CO_2 collected for isotopic analysis.

The $^{13}C/^{12}C$ ratios of expired CO_2 were determined with a Nier sixty-degree sector-type mass spectrometer. The mass spectrometer simultaneously determines the number of charged $^{13}CO_2$ and $^{12}CO_2$ molecules. To improve the sensitivity in measuring the $^{13}C/^{12}C$ ratio of a sample, which has very small quantities of ^{13}C, a standard source of CO_2 is used for comparison. In which case the recording instrument is electrically adjusted such that the standard is set equal to zero and, therefore, the ratio of the sample can be either on the positive or negative side of the zero position. Consequently, the $^{13}C/^{12}C$ ratio of a sample is reported as a value relative to the standard. The expression used to define this value is as follows:

$$\delta\ ^{13}C \text{ per mil} = \frac{^{13}C/^{12}C \text{ sample} - ^{13}C/^{12}C \text{ standard}}{^{13}C/^{12}C \text{ standard}} \times 10^3$$

The standard used was CO_2 derived from the fossil carbonate skeleton of Belemnitella americana. Since the $^{13}C/^{12}C$ ratio of the standard is relatively high, all δ values reported in this paper are negative. For example, the $\delta\ ^{13}C$ for atmospheric CO_2 is - 7 per mil. In this case, the $^{13}C/^{12}C$ ratio of the atmospheric CO_2 is 0.7 percent or 7 per thousand less than the ratio of the standard.

RESULTS

The $\delta\ ^{13}C$ per mil of rat lipid is less than that characterizing glycogen

(Table 1). The difference between the two δ values was expected since similar analyses of lipids and carbohydrates isolated from all organisms studied to date have yielded analogous results (3,4,5,7). When δ values of expired CO_2 were determined it was found that CO_2 samples collected from control rats had values most similar to that of glycogen. Furthermore, the δ values of expired CO_2 samples from alloxanized rats were decreased and approached the δ ^{13}C of rat lipids (Table 1).

Table 1. ^{13}C per mil of CO_2 from alloxanized rats and liver fractions.

	^{13}C per mil*
liver glycogen	-18.7
liver lipid	-23.3
respiratory CO_2-no treatment	-18.8
respiratory CO_2-alloxan treatment	-21.1

*Sample replication for respired CO_2 was ± 0.5 per mil and for CO_2 from combusted substrates was ± 0.2 per mil. The number of samples for respired CO_2 was in each case 4.

DISCUSSION

Previous research has shown that the δ value of respired CO_2 collected from plant tissue in different metabolic states reflects the δ value of the substrate being metabolized (3). Accordingly, from a comparison of the δ values of expired CO_2 with those values distinguishing the potential endogenous rate metabolites it can be concluded that alloxan treatment induces a change from carbohydrate to lipid degradation. In addition, it can be estimated from the data (Table 1) by conservation of mass equations (3) that untreated rats metabolize 2 percent lipid while alloxanized rats respire 52 percent.

Extrapolation of the results from rats to humans is most difficult but it should be pointed out that in theory the difference between the δ values

of CO_2 collected from non-fasting control and diabetic humans might even be greater than that found with rats for two reasons. First, net lipid synthesis is less in diabetics than in normal humans (8) and second, organisms that have lower quantities of lipids have larger differences between the δ values of their lipids and carbohydrates (5). Therefore, lipids in diabetic subjects should have a reduced $^{13}C/^{12}C$ ratio and consequently the δ value of CO_2 from diabetics would be much less than that from controls.

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REFERENCES

1. Stewart, G. A., and T. Hanley, in: Oral Hypoglycemic Agents, ed. G. D. Campbell (Academic Press, New York 1969) p. 348.

2. Hales, C. N., and P. J. Randle, Lancet i (1963) 790.

3. Jacobson, B. S., B. N. Smith, S. Epstein, and G. G. Laties, J. Gen. Physiol. 55 (1970) 1.

4. Jacobson, B. S., G. G. Laties, B. N. Smith, S. Epstein, and B. Laties, Biochim. Biophys. Acta 216 (1970) 295.

5. Park, R., and S. Epstein, Plant Physiol. 36 (1961) 133.

6. Park, R., and S. Epstein, Geochim. Cosmochin. Acta 21 (1961) 110.

7. Smith, B. N., and S. Epstein, Plant Physiol. 46 (1970) 738.

8. Garland, P. B., and P. J. Randle, Biochem. J. 93 (1964) 678.