THE ROLE OF COENZYME Q ON GLUCOSE OXIDATION AND LIPOGENESIS OF RATS IN VIVO AND IN VITRO

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Coenzyme Q (Co Q) is one of the lipid soluble substances with quinone nucleus, which distributes widely in animal, plant, and microorganism kingdoms including human.

It is well known that Co Q is localized between flavoprotein and cytochrome C_1 in mitochondria as a cofactor of electron transport system (Lester et al., 1958; Basfold and Green, 1959).

From the point of mechanism of Co Q action, it is possible that there would be a stimulation of glucose oxidation in tissue cells, Hoskin (1964) reported that menadion which contains a chemical structure similar to Co Q, increased the $^{14}C\ O_2$ output from glucose-1-^{14}C in in vitro experiments using nervous tissue of lobster.

The present study deals with an insulin-like action of Co Q_7, one of the Co Q compounds, which was found by the authors to stimulate glucose oxidation and fatty acid synthesis in rat adipose tissue in vitro and glucose oxidation in alloxan diabetic rats in vivo.

Materials and Methods

Male albino rats (Wistar strain) weighing 200 to 300 g were fed ad libitum until the time of sacrifice. Epididymal adipose tissues were prepared as

described by Martin (1958) and incubated at 37°C for 2 hours with shaking in a Krebs bicarbonate medium (1932) which was gassed with 95% O_2 - 5% CO_2. Glucose -1-^{14}C or glucose-6-^{14}C (0.2 μc), and non-radioactive glucose were present at a concentration of 100 mg/dl, insulin at a concentration of 10^{-3} or 10^{-4} u/ml, and Co Q_7 at a concentration of 50 or 200 ug/ml. Glucose content in the medium was determined by the method of Somogyi-Nelson (1952). CO_2 liberated by sulfuric acid was absorbed in 3 N NaOH for measuring ^{14}C activity. Fatty acids in adipose tissue were separated and assayed for ^{14}C activity by the method of Entenman (1957).

Male rats weighing about 150 g fasted for 18 hours were used for the experiments except for adipose tissue. Diabetes mellitus was produced by the intraperitoneal injection of 190 mg of alloxan per kg body weight. The diabetic rats were used seven or more days after injection and had blood glucose levels ranging between 200 and 500 mg/dl in the fed state.

40 mg of Co Q_7 per kg in surfactant was injected intramuscularly 18 and 42 hours before experiment. 10 μc of glucose-U-^{14}C or glucose-1-^{14}C in trace amount was injected intraperitoneally and then C O_2 expired was absorbed in 1 N NaOH for measuring ^{14}C activity.

Results and Discussion

As shown in Table 1, Co Q_7 in each concentration added *in vitro* did not increase glucose uptake but increased ^{14}C incorporation into CO_2 and fatty acids from glucose-1-^{14}C and glucose-6-^{14}C both in adipose tissue. Considering the results obtained from these experiments the ratio of Embden-Meyerhof pathway and hexosemonophosphate shunt was calculated by method of Winegrad and Renold (1958), and it was found that Co Q_7 stimulated both glycolytic pathways.

Insulin added *in vitro* stimulated the glucose uptake and the conversion to CO_2 and fatty acids from glucose. The striking difference of actions between Co Q and insulin was the effect on glucose uptake.

Table 1. Effect of Coenzyme Q_7 on ^{14}C Incorporation into CO_2 and Fatty Acids from Glucose-1-^{14}C or Glucose-6-^{14}C in Adipose Tissue of Rats *in vitro*

Additions	No. of Cases	Glucose Uptake	Glucose Oxidation and Lipogenesis from Glucose-1-^{14}C		Glucose Oxidation and Lipogenesis from Glucose-6-^{14}C		Via hexose-monophosphate shunt
			CO_2	Fatty Acids	CO_2	Fatty Acids	
		μ moles/g wet weight	μ moles/g wet weight		μ moles/g wet weight		%
None**	9	9.50 ± 0.72*	1.46 ± 0.08	1.15 ± 0.04	0.44 ± 0.01	2.75 ± 0.29	58***
Co Q_7 50 μg/ml	9	10.78 ± 1.10 n. s.	2.31 ± 0.17 p<0.01	1.74 ± 0.20 p<0.05	1.29 ± 0.22 p<0.01	4.93 ± 0.23 p<0.01	64
Co Q_7 200 μg/ml	9	10.61 ± 1.10 n. s.	2.36 ± 0.13 p<0.01	1.62 ± 0.13 p<0.01	1.18 ± 0.17 p<0.01	5.48 ± 0.32 p<0.01	70
Insulin** 10^{-4} U/ml	6	14.28 ± 1.89 p<0.01	2.52 ± 0.45 p<0.05	1.86 ± 0.26 p<0.01	0.58 ± 0.09 n. s.	5.06 ± 0.73 p<0.01	63
Insulin** 10^{-3} U/ml	6	20.60 ± 2.45 p<0.01	4.65 ± 1.70 p<0.01	4.41 ± 0.46 p<0.01	0.44 ± 0.03 n. s.	10.30 ± 1.24 p<0.01	57

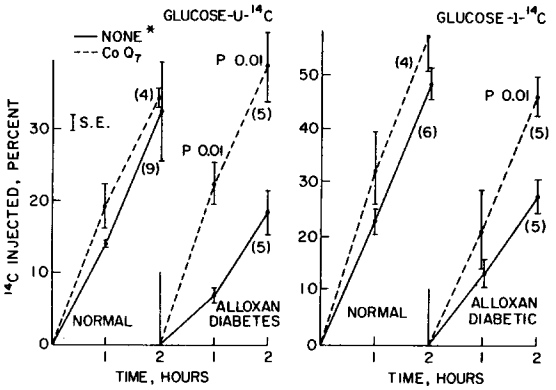
* Means ± standard error. n. s. means "not significantly different from control".

** The same amount of surfactant to Co Q_7 was added.
Rat epididymal adipose tissues were incubated at 37°C for 2 hours with shaking in a Krebs bicarbonate medium (1932), including radioactive and nonradioactive glucose at a concentration of 100 mg/dl.
Glucose was determined by the method of Somogyi-Nelson (1952). ^{14}C activities of $BaCO_3$ obtained from liberated CO_2 and fatty acids in adipose tissue were measured by Nuclear-Chicago Gas Flow Counter and the corrections on self-absorption factor were made.

*** % via hexose monophosphate shunt (Winegrad and Renold, 1958)

$$= \frac{(^{14}C \text{ fatty acids from glucose-6-}^{14}C) - (^{14}C \text{ fatty acids from glucose-1-}^{14}C)}{(^{14}C \text{ fatty acids from glucose-6-}^{14}C)}$$

Fig. Effect of Coenzyme Q_7 on $^{14}CO_2$ Output in Breath after Administration of Glucose-U-^{14}C or Glucose-1-^{14}C in Normal and Alloxan Diabetic Rats



*The same amount of surfactant to Co Q_7 was injected. 40 mg of Co Q_7 per kg was injected intramuscularly 18 and 42 hours before experiments. 10 μc of labeled glucose in trace amount was given intraperitoneally. ^{14}C activity of Ba CO_3 obtained from expired CO_2 was measured by Nuclear-Chicago Gas Flow Counter and the corrections on self-absorption factor were made.

Figure 1 showed the action of Co Q on glucose oxidation in in vivo experiments. ^{14}C output from alloxan diabetic rats was markedly decreased during the first 2 hours after giving glucose-U-^{14}C or glucose-1-^{14}C. After the Co Q_7 treatment for two days (18 and 42 hours beforehand), $^{14}C\ O_2$ output was not decreased below the normal range. In the same experimental condition, 1 u of regular insulin per rat given intraperitoneally 2 hours before isotope injection to alloxan diabetic rats caused a recovery of the $^{14}C\ O_2$ output nearly to the level of normal rat in the few cases, as did Co Q. In normal rats Co Q_7 did not have any influence on the $^{14}C\ O_2$ output.

The Co Q level per g wet weight of liver has been found (Shigeta and Izumi, 1965 a) to be decreased in steroid diabetic rats but was not significantly different from normal in animals with alloxan diabetes. After fractionation, the content of Co Q in liver mitochondria decreased significantly in

rats with steroid or alloxan diabetes, while there was no decrease in the latter treated with insulin (Shigeta and Izumi, 1965 a). These levels were parallel to the changes in succinic dehydrogenase activity in the liver. On the other hand, the Co Q content of the other fractions increased in rats with alloxan diabetes.

Crane (1961) reported that after acetone treatment of beef heart mitochondria Co Q_7, Co Q_8, Co Q_9 or Co Q_{10} could recover succinoxidase activity, although other Co Q compounds with shorter side chain could not. The authors (Shigeta and Izumi, 1965 b) found that Co Q content in rat liver and its mitochondria increased after the administration of Co Q_7, Co Q_9 or Co Q_{10}, but did not increase after that of Co Q_2 or Co Q_6. At that time the corresponding Co Q increased by paper chromatography after the administration of each Co Q compound. Therefore, it may be concluded that the injected Co Q could have been directly responsible for the metabolic effects on glycolysis and lipogenesis

Summary

Co Q_7 promoted CO_2 output and fatty acids synthesis from glucose taken up in epididymal adipose tissue of rats. In alloxan diabetic rats the decreased $^{14}CO_2$ output from injected ^{14}C-labeled glucose was recovered to normal level after the Co Q_7 treatment.

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