DETERMINATION OF GLYCOGEN TURNOVER RATES OF THE LIVER, CARDIAC MUSCLE AND SKELETAL MUSCLE OF THE DOG BY "C^{14}-GLUCOSE DILUTION METHOD"

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Several series of exreriments by Stetten, et al., [1,2] present current concepts of glycogen turnover in the amimal body. In their earlier study, the turnover rate of glycogen in vivo was estimated from the measurement of stably bound deuterium appearing in glycogen; the D_2O concentration in the body water was maintained by a D_2O conatant infusion method. This study using the rat showed that deuterium concentration of liver and carcass glycogen increased with time. Assuming the glycogen turnover to be metabolically homogenous, the turnover rates for liver glycogen and carcass glycogen were calculated to be 68% and 1% per day, respectively. In subsequent experiments employing C^{14}-glucose, however, Stetten and Stetten[2,3] reported that peripheral tiers of glycogen degraded enzymatically show more radioactivity than the less acce ssible limit dextrin within the core of the glycogen molecules. These results support the heterogeneity of glycogen turnover. The earlier calculation by D_2O based on the assumption of homogeneity must be revised.

Lorber, et al.,[4] postulated in their experiment that blood sugar must be the principal precursor for cardiac glycogen and that the incorporation of labeled carbon into cardiac glycogen in the intact amimal must be secondary to the formation of blood sugar from administered labeled compounds. On the basis of this assumption, Rhee, et al[5]. calculated the turnover rate of the cardiac glycogen in the isolated dog heart using the C^{14}-glucose constant; infusion method. In this experiment, the concentration of C^{14}-glucose and blood sugar in the perfusion system was maintained constant by secondary injection of additional labeled glucose. The reported turnover rate was 6.4% per hour for cardiac glycogen which was much higher than earlier reported values measured by D_2O in liver and carcass glycogen in rats.

The quantitative measurement of the glycogen turnover rate by tracer techniques has always inco rporated a constant tracer infusion method for assuring the steady incorporation of the tracers from the blood into tissue glycogen This technique requires detrmination of the characteristic disappearance of a single injection of the tracer to calculate the regulation of the infusion so as to maintain a constant concentration of the tracer during the experiment. Even so, it is difficult to maintain a conatant concentration. Data from the single injection method has in the past represented only qualitative aspects of glycogen turnover. This paper presents, however, the quantitative calculation of the turnover rate after a single injection of C_{14} glucose; Fick's principle as utilized in the indicator dilution method" for determining cardiac output is applied. This method is referred to as the "C^{14}-glucose dilution method".

THEORY

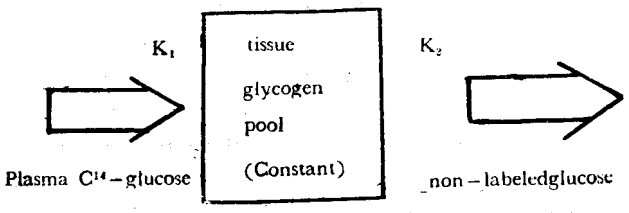
The calculation of the glycogen turnover rate utilizing the "C^{14}-glucose dilution method" is based on the following assumptions:

1) The principal precursor for tissue glycogen is plasma glucose as postulated by Lorber, et al. [4]

2) The tissue glycogen pool is constant during the experimental period. It has been known for many years that the concentration of circulating glucose is remarkably constant in the postabsorptive state. If there is a steady state of glycogen turnover, it is reasonable to suppose that the glycogen pool of the skeletal and cardiac muscles are constant in the postabsorptive state. The glycogen level in the isolated beating dog heart was demonstrated to be relatively constant before and after an

experimental period of 3 to 4 hours.[5] It is assumed that the glycogen pool in the isolated beating dog heart remains constant throughout the experimental period. It is doubtful, however, that a constant glycogen pool exits in liver even in pos tabsorptive state, since liver glycogen is the main source of blood sugar.

3) After a single injection of C^{14}-glucose, incorporation of plasma C^{14}-glucose into glycogen is steady as is the release of non-labeled glucose from glycogen for the period of the experiment.(Fig. 1)



k_1=Turn over rate into glycogen or in put rate.
k_2=Turn over rate from glycogen or out put rate.

<FIGURE 1> **Diagram of mode of steady glycogen turn-over after C^{14}-glucose single injection.**

It is difficult to prove that the released glucose is non-radioactive. Feller, et al.[6] reported that after a single injection of C^{14}-glucose, concentration of the plasma labelled glucose decreased exponentially with time, thus demonstrating the kinetic charact-eristics of a single homogeneous phase. These results support the concept that non-labeled glucose is released to the blood stream from liver glycogen for maintenance of a constant blood sugar conecentration. Even if labeled glucose is released from glycogen during the experimental period, the amount is negligible since incorporation of the radioactivity into glycogen from the plasma C^{14}-glucose is a minor fraction compraed with that of the injected plasma C^{14}-glucose, and released C^{14}-glucose from glycogen is directly proportional to the instantaneous radioactivity of tissue glycogen. On the basis of assumptions described above, the following calculation was made for the input rate (Kin Fig. 1). of plasma C^{14}-glucose into tissue glycogen after a single injection of C^{14}-gluc ose:

$$\frac{dn}{dt}=K_1(SA)_{PG}-K_2(SA)_G \cdots\cdots\cdots\cdots(1)$$

where

n: Total radioactivity of tissue glycogen or amount of C^{14}-glucose incorporated into glycogen at the given time.

t: Time.

K_1: Input rate constant of C^{14}-glucose into glycogen.

K_2: Output rate constant of C^{14}-glucose from glycogen.

$(SA)_{PG}$: Specific activity of plasma glucose or concentra-tion of C^{14}-glucose in the plasma glucose.

$(SA)_G$: Specific activity of glycogen or concentration of C^{14}-glucose in the tissue glycogen.

Integrating equation(1),

$$n=K_1\int(SA)_{PG}\,dt-K_2\int(SA)_G\,dt\cdots\cdots(2)$$

In equation(2), the first term represents total radioactivity incorporated into glycogen or total amount of the C^{14}-glucose incorporated in tissue glycogen and the second term represents released C^{14}-glucose from glycogen.

According to the third assumption,

$$K_2\int_o^t(SA)_G\,dt=0$$

Therefore, equation (2) is simplified as following,

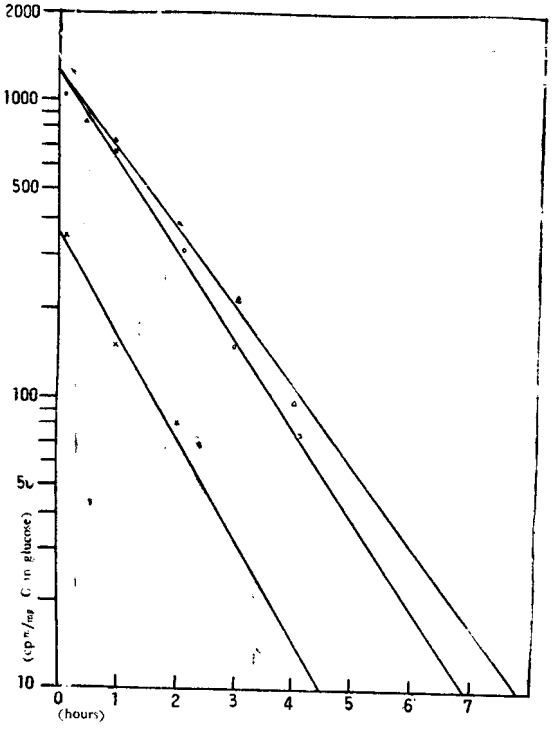
$$n=K_1\int_o^t(SA)_{PG}\,dt\cdots\cdots\cdots\cdots\cdots\cdots(3)$$

Rearranging equation (3),

$$K_1=\frac{n}{\int_o^t(SA)_{PG}\,dt}\cdots\cdots\cdots\cdots\cdots(4)$$

The numerator of equation (4) corresponds to the total radioactivity of tissue glycogen at the given time after a single injection of C^{14}-glucose and it can be easily measured. The denominator of equation (4) also can be calculated by planimetry, if we plot the $(SA)_{PG}$-time curve of plasma glucose after a single injection of C^{14}-glucose. Area under the $(SA)_{PG}$-time curve corres-ponds to the denominator, $\int_o^t(SA)_{PG}\,dt$ (Fig. 2 & 3)., a sample calculation is as follow: In dog 2, specific activity of the cardiac glycogen was 110 counts per minute per mg. carbon at the end of the experiment. Total counts incorporated per 100mg. of cardiac glycogen (n) can be calculated: $110\times100\times\frac{72}{162}=4889$ counts per minute. The area of the dilution curve is 1990 c.p.m./mg. C x hr. The turn-over rate K is $\frac{4289\text{ c.p.m.}}{1990\text{ c.p.m./mg. C x hr}}=2.46$ mg. C/hr., thus glu

cose containing 2.46 mg. of carbon is replaced every hour for 100 mg. of cardiac glycogen. Therefore $2.46\times\frac{162}{72}=5.6$ mg. of glucose is incorporated into 100 mg. of cardiac glycogen per hour. The turnover rate is 5.6%/hr.



<Fig. 2> SA-time curves of plasma glucose.

Best eye fitting straight lines are obtained on the semilogarithm in each dog, representing single homogenous disappearance of the labeled glucose from plasma.

×—×; Dog 1
○—○; Dog 2
△—△; Dog 3

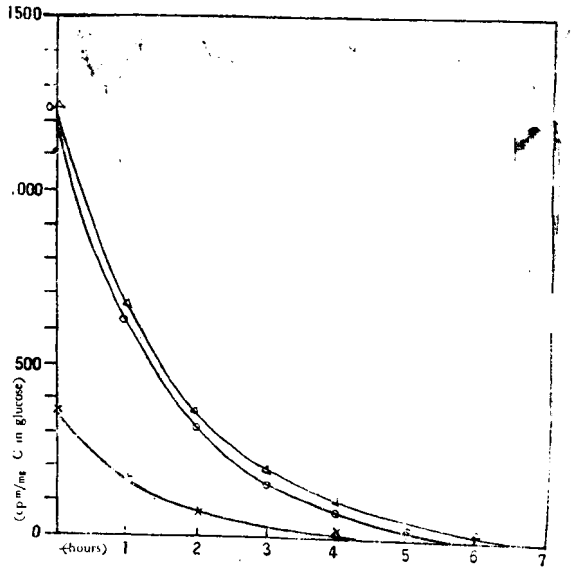
EXPERIMENTAL METHODS

C^{14}-glucose intravenous solution: 100 uc. of universally labeled glucose (Isotope Specialties Company) is diluted in 100 cc. of normal saline. A single injection, 20 uc or 20 cc of this solution, is injected via the jugular vein of the dog.

Amimals: Adult normal dogs in a fasting state for at least 12 hours were used. Their weight ranged from 12.8-15.4 Kg. All dogs were given nembutal (30 mg/Kg).

Analytical method: The following chemical procedures were employled in this series of experiments. For plasma gucose, the combined method of Somogyi (7) and Nelson (8); for estimation of the tissue glycogen, a modification of the procedure of Good, Kramer, and Somogyi (9); for isolation of the glycogen, the method of Stetten and Boxer (1); for total carbon, the method of Van Slyke-Folch (10) were used.

Radioactivity method: For specific activity of



<Fig. 3> SA-time curves of plasma glucose.

These curves are rearranged on normal section graph paper after drawing Fig. 2. Areas under these curves correspond to the numerator of equation (4)

×—×; Dog 1
○—○; Dog 2
△—△; Dog 3

plasma glucose. plasma glucose was precipitated to phenylglucosazones, which were degraded to total carbon and precipitated to barium carbonate on the Whatman No.542 filter papers (11). The mounted barium carbonate was counted as infinitely-thin samples with an end window Geiger-Müller counter(Tracer Laboratories, Inc.). The specific activity of isolated glycogen was measured in the same manner as phenylglucosazone.

General procedures: For blood sampling, the jugular vein of the dog was canulated. After a single injection of C^{14}glucose, hourly blood samples were collected for plasma glucose level and specific activity of plasma glucose determinations for a period of 5-6 hours. At the end of the experiment, skeletal and cardiac muscles and liver tissue were quickly excised for glycogen determination

RESULTS AND DISCUSSION

Initial accelerated labeled glucose in plasma by injection of C^{14}-glucose decreased exponentially with time. the SA-time curve of plasma glucose was a straight line when plotted semilogarithmically as shown in Figure 2. This is good experimental evidence depicting the kinetic characteristics of the disappearance of plasma C^{14}-glucose in the sterdy state. It can be predicted that the incorporation rate of plasma C^{14}-glucose into tissue glycogen will be decreased with time since the incorporation rated is directly proportional to SA of plasma glucose in the single homogeneous phase as shown in the SA-time curve of plasma glucose. The straight line of the SA-time curve on semilogarithm paper was extrapolated to a time axis and the time of complete disappearance of plasma C^{14}-glucose from the body after a single injection of C^{14}-glucose was determined, thereafter this curve was rearranged to a linear graph (Fig. 3) and planimetry was done by the weighing method.

<TABLE 1> GLYCOGEN TURN OVER RATE

Tissue	Dog	glycogen concentration mg/100 gm tissue	S.A of glycogen cpm/mgC	Total cts in glycogen △ cpm/100 gm tissue	cpm/100 mg glycogen	area of dilution curve ○ cpm/mg-C hr	glucose incorporated in glycogen ◎ mg/hr/-100gm tissue gly	glucose release from glycogen mg/hr/-100gm tiss gly.	glucose incorporated in 100mg glycogen ● mg/hr/-100mg gly.	glucose released from100. mg glycogen mg/hr/-100mg gly.	turn over rate (K) %/hr	$t_{\frac{1}{2}}$ hr
heart glycogen	1	440	29	5,670	1,290	430	33	29.7	7.5	6.75	6.75	10.3
	2	430	110	21,000	4,889	1,990	26.4	23.8	6.14	5.51	5.51	12.6
	3	667	90	26,600	3,980	1,750	38.0	34.2	5.7	5.1	5.1	13.5
								29.2	6.44	5.8	5.8	12.5
	mean						32.5					
muscle glycogen: (diaphragm)	1	162	10	720	445	430	4.2	3.8	2.59	2.33	2.33	29.8
	2	303	52	7,000	2,310	1,990	8.8	7.9	2.90	2.61	2.61	26.5
	3	453	67	13,500	2,980	1,750	19.3	17.3	4.3	3.81	3.81	18.1
								9.66	3.26	2.92	2.92	24.8
	mean						10.76					
liver glycogen	1	980	—	—	—	—	—	—	—	—	—	—
	2	176	215	16,800	9,550	1990	21.2	19.0	12.0	10.8	10.8	6.4
	3	133	248	14,650	11,000	1750	20.9	18.8	15.7	14.3	14.3	4.9
	mean						21.1	18.9	13.9	12.6	12.6	5.7

△ Total cts in glycogen=SA of glycogen (cpm/mg. C)×mg. of glycogen×72/162

○ Area of C^{14}-glucose dilution curve (SA-time curve of plasma glucose)

● This column calculated by Fick's equation as follow :

n(total cts./min.)/∫(SA) dt=5 th or 6th column/7 th column×180/72

× $t\,1/2=0.693/$turn over rate(K)

Table 1. summarizes the experimental date for the turn-over rates of glycogen in liver, cardiac tissues and in skeletal muscle in three normal dogs. The glycogen concentration in the various tissues of the three dogs is variable. The specific activity of glycogen, however, as shown in the fourth column, demonstrates a relatively constant ratio between the three tissues in each dog. A quantitative value of the glycogen turn-over rate for liver, cardiac, and skeletal muscle glycogen can be arrived by equation (4). From this equation, average of 12.6% for liver glycogen, 5.8% for cardiac glycogen and 2.9% for skeletal muscle glyecgen is turn-overed every hour The time for turn-over of half of each tissue glycogen pool (half time or $t\frac{1}{2}$) is 5.7 hours for liver, 12.1 hours for heart and 21.5 hours for skeletal muscle glycogen. These values represent much faster turn-over rates than the values reported by Stetten and Boxer in the rats.[1] Cardiac glycogen turn-over rates compared favorably with data obtained by Rhee, et al.,[5] using the constant C^{14}-glucose infusion method. Direct calculation is employed to obtain the turn-over rate, the ratio of specific activity of plasma glucose to that of glycogen is divided by the experimental time period. This method was believed to be best

for estimating the glycogen turn-over rate in the steady state. Earlier determinations with D_2O by Stetten and Boxer[1] supposed a homogeneous in corporation of D_2O into glycogen; however, since D_2O incorporation into glycogen is secondary to the formation of a precurssor which carries D_2O, it is questionable whether homogenous incorporation occurs. In view of the consistent turn-over rates of cardiac glycogen determined by both the C^{14}-glucose dilution method and the constant C^{14}-glucose intusion method, we can execpt similar results in liver and skeletal muscle glycogen turn-over rates.

It is interesting that the turn-over rate of liver glycogen is about two times faster than that of the cardiac glycogen and four times faster than that of the skeletal muscle glycogen, thus liver glycogen is metabolically more active than cardiac and skeletal muscle glycogen.

SUMMARY

Calculation of the glycogen turn-over rate by "C^{14}-glucose dilution method" following a single inj-ection of C^{14}- glucose is presented.

Turn-over rates of liver, cardiac and skeletal muscle glycogen in the normal dog were 12.6%/hr, 5.8%/hr and 2.9%/hr, respectively.

Values calculated by the "C^{14}-glucose dilution method" for cardiac glycogen compared favorably with values obtained in the isolated dog heart using the constant C^{14}-glucose infusion method.

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—國 文 抄 錄—

C^{14}-포도탕 희석법으로 인한 개의 각조직 glycogen의 교체률 측정에 관한 실험

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이상돈 · 남기용

G^{14}-포도탕의 닫일주입법으로 인한 조직 glycogen의 혈장포도탕과의 "교체률"(turnover rate)의 수량적 측정에 관한 원리를 소개하고 이 방법을 C^{14}-포도탕 희석법이라고 칭 하였다,

위의방법을 이용하여 개의 간장, 심장 및 횡문근 glycogen의 "교체률"을 측정 한 결과 간장 glycogen에서 12.6%/hr, 심장에서 5.8%/hr, 횡문근에서 2.9%/hr의 값을 얻었다. 즉. 간장 glycogen의 "교체률"는 심장 glycogen의 2배, 횡문근 glycogen의 4배 가량 빠름을 볼 수 있으며, 이러한 각조직 glycogen 사이의 "교체률"의 차이는 간장 glycogen이 대사과정에서 가장 활발함. 을증명하였다.

위의방법으로 측정한 glycogen의 교체률의 값과 „계속C^{14}-포도탕주입법"으로 측정한 값과 비교 한 즉 근사한 값을 얻은 사실로보아 "C^{14}-포도탕 희석법 이 glycogen 교체률측정에 "계속주입법" 보다 간편하고 신빈성이 있음을 논의 하였다.