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Biokinetics of Carbohydrate and Lipid Metabolism in Normal Laying Hen.

II. Incorporation of Activity in Plasma Lipid After Injection of Glucose-U-C^{14}

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正常產卵鷄에 있어서 炭水化物과 脂質代謝의 生動力學

(第二報) 放射能(C^{14})포도당 注射後 血漿 脂質로의 放射能의 併合

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SUMMARY

The incorporation of activity in plasma lipid was determined after injection of u-C^{14}-glucose to normal laying hen. The extraction of total lipid from plasma was carried out by the method of Floch et al. with some modifications. The counting of carbon-14 activity was conducted by a Beckman Liquid Scintillation System, The concentration of plasma lipid was estimated as much as 3,070mg per 100ml of plasma taken at 5 minutes after injection of tracer amount of u-C^{14}-glucose. The specific activity per gram of plasma lipid carbon atom per injected dose per kg body weight increased gradually following the time after injection until 120 minutes, whereas the glucose activity decreased logarithmically. The partial incorporation quotient of activity in plasma lipid from that of glucose was 0.73% until 120 minutes after the injection.

INTRODUCTION

It is well known that glucose is broken down to form triose which is oxidized and produces carbon dioxide and acetic acid. The latter associates with coenzyme A to produce acetyl coenzyme A[1]. These reactions occur aerobically in the living cell. This acetyl coenzyme A contributes to produce many kinds of fatty acids through malonyl coenzyme A, long chain acetyl coenyme A and phosphatadic acid. Hence some of the lipids are derived from glucose.

At the previous investigation of turnover of glucose,[2] it was shown that the disappearence of activity in plasma glucose was logarithmically rapid. Probably some of the glucose would be turned to lipid and the incorporation of activity into plasma lipid from u-C^{14}-glucose activity might be happened. And some of the lipids pro-

duced in living cells would recycle in blood.

In this experiment the figure of incorporation was observed after injection of u-C^{14}-glucose into a hen. The plasma samples used in this trial were those taken at previous experiment on glucose turnover[2].

French and Popjack[7] compared glucose and acetate as a precursor of milk fat in rabbits. Gibbs et al.[8] investigated the mechanism of bacterial fermentation of glucose into lactic acid using u-C^{14}-glucose. Muller and Davis[12] experimented the incorporation of acetate-1-C-C^{14} intofatty acids in blood of bovine. Riis and Herstad[14] measured the incorporated ratio into lipid in normal laying hens. Riis[15] determined the ratio in swine also.

In this experiment only total lipid in a hen's plasma was analyzed for the concentration and the without any measurement of individual fatty acid or of lipid's kind.

EXPERIMENTAL METHODS

1. Isolation of total lipid.

Dole[3] used isoprophyl alcohol and heptane as the extracting solvents for lipid. Folch et al.[6] isolated the lipids from animal tissues using chloro-form-methanol mixture. Riis and Herstad[14] used the latter, too. Here, also, the latter was used for extraction of lipid. Dole[3] prepared a solution of IN-H_2SO_4 for washing of crude extract. Floch et al.[6] used 0.7% NaCl solution for this purification. On this experiment the latter solution was applied. After suction out of upper phase, a mixture of chloroform, methanol, was water(3:48:47) was poured in to wash the crude fat further. Folch et al. observed this ratio of mixture in uppersolvent phase by allowing to stand the mixture of chloroform, methanol and water(8:4:3).

Folch et al.[6] however, did not experiment on the pH of the solution. But Dole[3] described the the presence of strong acid in law concentration had no significant effect on the phase volume relations, but neutral mixture made removal of neutral fat and dissociated some of fatty acids in $RCOO^-$ and H^+, which ions were removed through the upper phase. Here, phosphate buffer solution (pH 5) was used to lower the pH. The 25ml of 2:1(volume) chloroform-methanol mixture and 1 ml of phosphate buffer were placed in 100 ml Erlenmyer falsk. One ml of plasma kept in a freezer and dissolved in room temperature was pipetted into above flask, which was covered with cork stopper and stirred occasionally on the table for one hour.

After this extraction the solution was filtered into a high tube, which had 9ml 1% NaCl solution and marked at the volume of 45ml, previously. The flask was rinsed with small amount of chloroform-methanol mixture two times and the filter paper and the end of funnel was rinsed carefully. The 50 mg D(+)-glucose was added as a carrier of radioactive glucose, a suitable amount of chloroform-methanol was added to reach full mark of 45ml. Covered and mixed tubes were placed in refrigerator for 2 hours.

Then the tubes were unplugged and the upper phase was removed away by a suction. A suitable amount of mixed solution (chloroform 3+methanol 48+0.7% NaCl-water solution 47) was added to make 45 ml. After mixing, the tubes were placed in the refrigerator again for 2 hours. The upper phase was suctioned out, the lower phase was poured into 100ml Erlenmyer flask, and the tubes were rinsed with a small amount of methanol twice.

2. Determination of lipid content and activity.

Three beads of glass balls were added in the flask to prevent boiling up. The flasks were placed in a vacuum oven(45°C) under CO_2 gas overnight for evaporation of solvents. After heating the flasks were cooled in room temperature, 5ml of petroleum ether was added, covered with cork stoppers, mixed and placed on the table to dissolve the lipid for one hour. The solution was filtered through a cotton funnel into a dried and weighed vial to obtain a clear solution. The cotton funnel was obliged to test for the tightness previously, i. e., petroleum ether had to fall drop

by drop. The lipid of the Erlenmyer flasks were rinsed with small quantity of petroleum ether everytime after pouring.

The Erlenmyer flask, the funnel, and the end of the funnel were rinsed carefully. The vials were placed in a vacuum oven overnight to evaporate the solvent. After cooling in a desicator the weight was measured. The difference between this weight and vial's weight gave the amount of total lipid in plasma samples. The 10ml of 0.5 % PPO toluene solution (Bray)[1] was added to this vial, after covering and mixing the vials were placed in a dark room overnight.

The activity of this total lipid was measured using Beckman LS-100 Liquid Scintillation System (Ehler)[4]. Before the major counting maximum gain was obtained for first sample. In order to get internal standard's efficiency of counting 50μl of 1-C^{14}-palmitic acid, toluene soultion was added after the counting of only lipid. Also next day the activity was counted, and calculated the calculated the specific activity for each sample.

RESULTS AND DISCUSSION

The lipid content in plasma decreased following the time after injection because of the blood taking as same as in glucose content. Determination of lipid concentration in plasma showed 3,070-2,190mg per ml. The specific activity of plasma lipid was plotted against to time after u-C^{14}-glucose injection on Figure 1. Viewing from this curve it was known that the incorporation of activity into plasma lipid increased gradually according to the time.

Even though whole figure of this incorporation was not known unfortunately, until 120 minutes the curve showed a tender sigmoid[5]. Using these plotting the regression between specific activity and time after injection was analyzed:

$$y=0.00097x-0.0033$$

The results of t-test indicated that this straight line had no significant deviation from the plottings (or from dotted line).

For a comparison between lipid and glucose

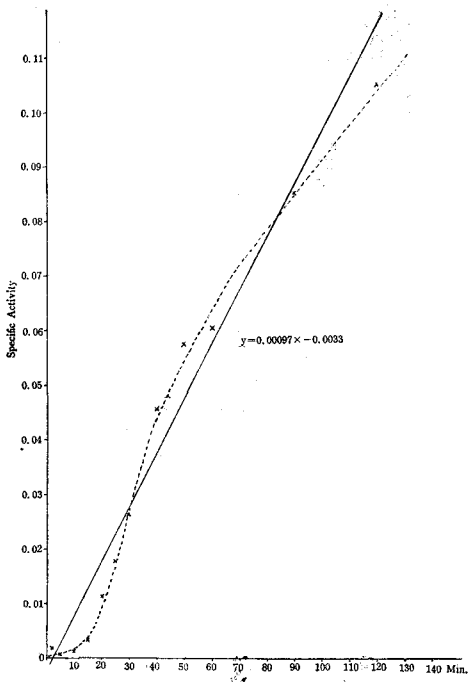
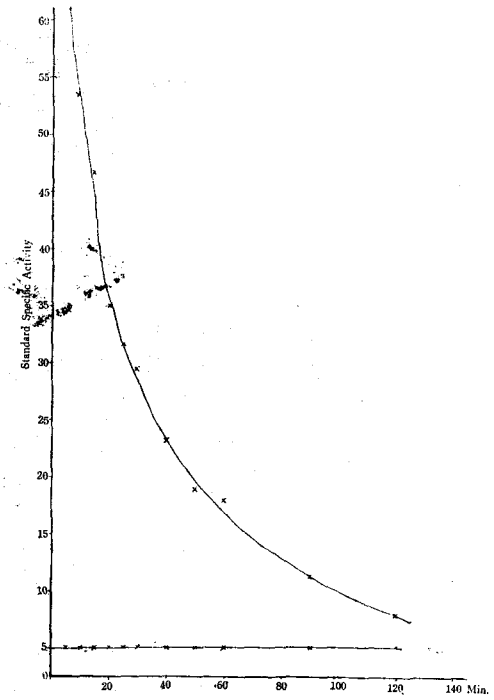


Fig. 1. Curves showing the incorporation of activity in plasma lipids after injection of u-C^{14}-glucose in a hen. Straight line is regression line and dotted line is a curve closing to the plots.

activity the specific activity was expressed in nCi per gram carbon atom per injected dose per kg body weight. The value thus obtained was called standard specific activity. Figure 2 shows the curves of standard specific activities against the time after injection for both glucose and lipid. There was great difference between the standard specific activities of glucose and those of lipid. However, the standard specific activity of fore intermediate decreased logarithmically, whereas those of latter increased in straight line. On Fig. 3, the logarithmic standard specific activity was plotted against the time after injection. The regression between logarithmic standard specific activity and the time (minutes) was analyzed for glucose only:

$$y=1.75-0.0106x$$

The picture on lipid showed a reversily standing logarithmic shape[15] and it was reconfirmed that the standard specific activities of lipid



Time after injection

Fig. 2. Curves showing the relationship between the standard specific avticity of plasma glucose(upperline) and lipid(Lower line) after injection of u-C^{14} -glucose in a hen.

increased linearly on Figure 2.

Kleiber[16] showed that so-called transfer quotient which defines the precursor-product relation ship could be expressed as following:

$$q=\frac{\int_0^{\infty} x'dt}{\int_0^{\infty} xdt}$$

Where x'=standard specific activity of product (plasma lipid)

x=standard specific activity of precursor (plasma glucose)

Provided that the "incorporation quotient" would be used here instead of "transfer quotient" and that "partial incorporation quotient" would be used for this experiment because of the short period, i.e., not ∞ time, but 120 minutes, the quotient would be:

$$q=\frac{\int_0^{120} x'dt}{\int_0^{120} xdt}$$

From Figure 2 the area under the straight line for lipid could be obtained easily:

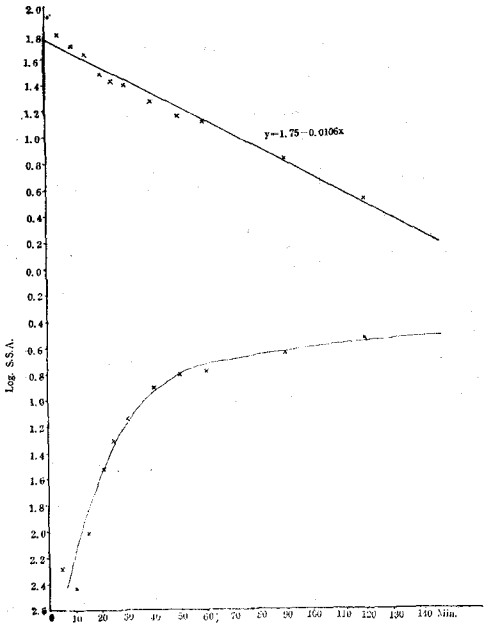
$$\int_0^{120} x'dt=[x'1/2t]_0^{120}$$
$$=0.279\times 1/2\times 120$$
$$=16.74$$

The area under the line for glucose could be gained from the zero time standard specific activity and slope of the straight line on Figure 3.

$$\int_0^{120} xdt=\int_0^{120} x_0e^{-dt}$$
$$=\left[\frac{x_0}{a}(-e^{-dt})\right]_0^{120}$$
$$=\frac{x_0}{a}$$
$$=2,290$$

The partial incorporation quotient would be:

$$q=\frac{16.74}{2,290}=0.0073=0.73\%.$$



Time after injection.

Fig. 3. Curves showing the relationship between the logarithmic standard specific activities of plasma glucose (upper line) and lipid (lower line) after injection of u-C^{14}-glucose in a hen.

The lipid concentration in plasma samples were 3,070 −2,190 mg per 100ml corresponding to 5−120 minutes after injection, but the value of

3,070mg could be selected for this hen's normal behaviour. Heald and Rookledge[10] published that the plasma lipid content was 1,500-3,000mg per 100ml at first egg production of the hens. This normally laying hen had a comparatively high amount of lipid in plasma.

Viewing from Figure 1 there was continous increase in specific activity until 120 minutes which was the final time for taking the plasma sample. Riis and Herstadt[14] experimented on the incorporation of activity into plasma lipid after injection of 17 and 10.5 μCi to the laying hens. The standard specific activity of plasma lipid was increased following the minutes until 60 minutes where the curve showed a peak. After that time there was a decrease until 360 minutes.

The trial on swine represented also same magnitude as on hens, according to the results of Riis[51]. If there were more investigations in this experiment the curve would show possible peak and decrease.

Althrough the procuct investigated for the transfer quotient by Riis and Herstad was CO_2 but not lipid, it was found that in nonfasted hens 30~35% of the CO_2 originated directly from oxidized glucose, whereas the percentage in fasted hens was only 16%. In this experiment the partial incorporation quotient into lipid from glucose was 0.7% only. Hence it was known that much amount of glucose would be used for direct oxidation to produce heat and CO_2 and very little amount might be used for the synthesis of lipids.

要　　約

均一하게 標識된 C^{14}-포도당을 産卵鷄에 注射한 後 血漿 脂質로 放射能이 併合되는 狀態를 調査하였다. 血漿에서 全脂質을 抽出하는 方法은 Folch 等의 方法을 조금 變型하여 適用하였으며 放射能의 測定은 液體신치레숀카운터에 依하여 實施하였다. 供試한 産卵鷄의 血漿 脂質의 濃度는 追跡子量의 C^{14}-포도당을 靜脈에 注射한 後 5分에 採取한 試料에서 100ml當 3.07g을 나타내었다. 포도당의 比放射能은 對數的으로 急速하게 減少하는 反面 kg體重當, 注射單位量當, 그리고 血漿 脂質의 그람 炭素原子當 比放射能은 注射後 120分까지 時間에 따라서 매우 徐徐히 增加하였다. 포도당의 放射能으로부터 血漿 脂質의 放射能으로 併合되는 率은 注射後 120分까지 0.73%이었다.

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