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Biokinetics of Carbohydrate and Lipid Metabolism in Normal Laying Hen

Part Ⅲ. Determination of Radiochemcal Purity of ^{14}C(U)-Glucose Soultion by Liquid Scintillation System Using Glucose Pentaacetate

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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

第三報, 五醋酸化포도당의 合成 및 液體신치레숀카운터에 依한 均一標識 ^{14}C- 포도당의 放射化學的 純度 測定

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Summary

The radiochemical purity of ^{14}C(U)-glucose solution to be injected to normal laying hen was investigated for studying biokinetics of carbohydrate and lipid metabolism. The liquid scintillation counter was employed for determining the activity of carbon-14. The barium hydroxide and zinc sulfate were adopted to precipitate the protein in the solution. The glucose content in the solution was observed as 0.912 mg per m*l*, applying Hultman's method. The specific activity of ^{14}C(U)-glucose solution was known as 31.3 nCi/mg glucose. The glucose pentaacetate was synthesized to isolate the pure glucose from the solution. The specific activity of pure glucose was measured as 28.5 nCi/mg glucose. Therefore, it was known that the radiochemical purity of the solution was 82.7%.

Introduction

Since ^{14}C is a radioisotope which emits beta ray having half life of 5,568 years, liquid scintillation counter is generally used for counting it's activity. Nuclear Enterprise, Ltd. doveloped 220 scintillator for aqueous solution of ^{14}C (Ford)[1] and toluene scintillator was employed by Bray[2]for non-aqueous solution. Diotal scintillator was used for hyamine solution which absorbed CO_2 gas expired by animal(Herberg)[3].

In order to deproteinize the plasma, Bierry and Gruzewska[3] utilized mercurous nitrate. Good et al[5]. employed nitric sulfate, and Somogyi[6] adopted barium hydroxide and zinc sulfate.

Somogyi[6] used copper phosphate for glucose

measurement, Hassid and Abraham[7] adopted sulfuric acid and thiourea. Acetic acid and o-toluidine were employed for developing blue color from glucose by Hultman.[8] Since some other nutrients were labelled with ^{14}C, pure glucose in plasma supernatant should be isolated for measuring specific activity of pure glucose. Various methods were used to achieve this objective. Feller et al[9] prepared a derivative of phenyl glucosazone, but the isolation of the derivative was difficult. Steele et al[10] converted the crude osazone to phenyl glucosotriazole which was easily purified, but the yield of purified product was of the order of 23%. Jones[11] published a method for determination of glucose activity using pentaacetate. This paper descirbes the results of measuring radiochemical purity of ^{14}C(U)-glucose solution by liquid scintillation counter using glucose pentaacetate.

Experimental Methods

1. Determination of activity in ^{14}C(u)-glucose solution to be injected to hen

The activity of ^{14}c(u)-glucose was measured by internal standard method, using channel 2 of Beckman Liquid Scintillation System. In order to get suitable activity for counting the solution to be injected was diluted 50 times (solution A). One-tenth m*l* of solution A and 10m*l* of NE 220 scintillator were mixed in a glass vial and stored overnight in a dark room. The background and total activities were counted. The 50m*l* of standard ^{14}C(U)-glucose solution was added into the vial. The second counting was carried out next morning.

2. Precipitation of protein and glucose determination

In order to get similar solution to plasma on the basis of glucose concentration the 18m*l* of stock glucose solution containing 0.998mg glucose per m*l* and 2m*l* of solution A were mixed in a test tube(solution B). The 2m*l* of 0.3N-$Ba(OH)_2$ and 1m*l* of solution B were placed in a centrifuge tube. Then 1m*l* of H_2O and 2m*l* of 0.75N-$ZnSO_4$ were added and mixed thoroughly(solution C). After centrifuging the clear supernatant was taken and used both for glucose determination and for preparation of glucose pentaacetate(GPA).

The Hultman's method was selected to measure glucose content. The standard curve was made with range of 0-0.16mg/0.5m*l*.

3. Isolation of glucose as pentaacetate and counting it's activity

The Jones procedure[11] was employed with some modifications. The 100mg carrier glucose was added to 3m*l* of supernatant in a lipless beaker. The solution was evaporated to a thick syrup, 60-70mg of ground sodium acetate and 1m*l* of acetic anhydride were added to acetylate the glucose. Beakers were placed in an oven of 105°C to acetylate chemically. The 8m*l* H_2O was added. The mixed solution was boiled on a heater until all oily globules disappeared. After adding charcoal the solution was heated again. The hot solution was filtered through a filter paper and hot funnel quickly.

The filtrate was collected in an Erlenmyer flask. It was cooled to crystallize the GPA. It was filtered through a glass filter by suction. The crystals on glass filters were dried in a vacuum oven (45°C). Those were weighed and transferred to vials. The 10m*l* of non-aqueous scintillator was poured in and the activity was counted as in the solution to be injected.

Results and Discussion

1. Determination of activity in ^{14}C(u)- glucose solution to be injected to hen

Mean activity was found to be 28.5 nCi for the diluted solution A(0.1m*l*). Thus, 25m*l* of solution A would have 7,125 nCi of activity, corresponding to 0.5m*l* ^{14}C(U)-glucose solution. Consequently the activity of 1m*l* injection solution would be 14.2μCi(Table 1).

Wang and Willis[12] published that a persistent problem in scintillation detection was "quenching." Quenching may occur by any of

Table 1. Activities of solution A.

Sample No.	C.P.M.	C.P.M. Corrected	Efficiency %	Activity nCi
1	42.304	42.280	63.5	28.5
1+St.9.45nCi	55.648	13.344	63.5	
2	41.353	41.330	64.9	28.7
2+St.9.45nCi	54.975	13.622	64.9	
3	41.212	41.190	65.1	28.3
3+St.9.45nCi	55.064	13.792	65.1	
Mean			64.5	28.5

several mechanisms; chemical, dilution, color, optical, etc. In order to gain efficiency, various techniques are employed; internal standard method, dilution method, external standard method, and so forth. In this trial the internal standard method was adopted and 63~65% efficiency was obtained in solution A. It was known that 95% efficiency could be obtained as a maximum value for ^{14}C. The possible reason for low efficiency in this experiment might be the water and the chemicals added to generate the fluor light.

2. Precipitation of protein and determination of glucose content

The five replicate 0.5m*l* supernatant solutions had the glucose as shown in Table 2. The average value of 0.076mg glucose per 0.5m*l* supernatant was obtained. Conseqently it was known that the 6m*l* solution C included 0.912mg glucose, provided that the solution C had the same concentration of glucose as had the supernatant. Since 6m*l* solution C was from 1m*l* solution B, the concentration of glucose in solution B was estimated as 0.912 mg per m*l*. Furthermore the one m*l* solution B corresponded to 0.1m*l* solution A. Therefore, the mean specific activity of solution A could be calculated as 31.3 nCi/mg glucose, because 0.1m*l* solution A had 28.5 nCi activity.

Table 2. Glucose concentration in supernatant and chemical recovery of glucose pentaacetate (GPA).

Sample No.	Clucose in 0.5m*l* supernatant (mg)	Glucose in 3m*l* supernatant (mg)	Glucose added as carrier (mg)	Total glucose (mg)	Theoretical yield of GPA (mg)	GPA recovered (mg)	Chemical recovery (%)	Glucose in GPA from 3m*l* supernatant (mg)
1	0.078	0.468	100.0	100.47	218.02	160.8	73.8	0.345
2	0.077	0.462	100.0	100.46	218.00	154.5	70.9	0.328
3	0.076	0.456	100.0	100.46	218.00	171.8	78.9	0.359
4	0.078	0.468	100.0	100.47	218.02	126.6	58.1	0.272
5	0.076	0.438	100.0	100.43	217.93	146.3	67.1	0.294
Mean	0.076	0.456	100.0	100.46	218.00	152.0	69.5	0.319

Since there was clear boundary between supernatant and precipitate in the process of deproteinization, it was easy to take supernatant for glucose determination. It was known that barium hydroxide and zinc sulfate were excellent reagents for precipitating protein. During heating tubes on water bath to develop blue color it was found that the rubber-stopper should be tightly closed. The stoppers were somtimes opened up due to the inner pressure. Blue color was generated readily. The standard curve was fairly linear. And it was thought that the acetic acid which was employed here and also by Hultman [8] was more convenient and less dangerous compared to sulfuric acid by Hassid and Abraham[7].

The specific activity of solution A was determined as 31.3 nCi/mg glucose. This solu-

tion was from the solution to be injected which had been stored in freezer for several months. Therefore, some degree of radiolysis could be assumed(Wang and Willis)[12].

3. Isolation of glucose as GPA and counting it's activity.

The weight of crystalized GPA and theoretical amount of GPA allowed to calculate chemical recovery as in Table 2. Absolute amount of glucose in GPA which was synthesized from 3 m*l* of supernatant could be calculated according to the recovery. The glucose weight was used for gaining specific activity as in Table 3.

The specific activity of glucose was observed as 25.89 nCi/mg. It was concluded that the purity of the solution to be injected was 82.7 % when compared to 31.3 nCi/mg obtained in article 1. Furthermore, the injection amount could be decided according to this purity. The more amount of injection solution than theoretical one should be taken.

Table 3. The activities of glucose pentaacetate (GPA)

Sample No.	C.P.M.	C.P.M. Corrected	Efficiency (%)	Activity (nCi)	Glucose (mg)	Spec, Act. (nCi/mg)
1	13.939	13.910	68.85	9.10	0.345	20.38
1+St.9.45 nCi	28.515	14.485	68.85			
2	13.834	13.810	71.71	8.68	0.328	26.41
2+St.9.45 nCi	28.894	15.060	71.71			
3	14.000	13.970	69.57	9.50	0.359	25.21
3+St.9.45 nCi	28.609	14.609	69.57			
4	10.893	10.850	69.50	7.03	0.292	25.82
4+St.9.45 nCi	25.467	14.594	69.50			
5	11.443	11.420	68.43	7.51	0.294	25.54
5+St.9.45 nCi	25.813	14.370	68.43			
Mean			69.61	8.27	0.319	25.89

Jones[11] used glucose pentaacetate for gaining radiochemical purity. Knowing purity is an essential step prior to carrying out an in vivo experiment. Since the supertantant had little amount of ^{14}C(u)-glucose the carrier glucose was added. The glucose, acetic acid, and acetic anhydride were reacted to prepare GPA. However, there were some difficulties in the process of GPA crystallization. In order to evaporate water and acetic acid the sample beaker was placed on gas burner. When overheated, it sprang up and was spoiled. The analyst should take care to put the beaker away before overheating.

Riils and Herstad[13] injected 4 to 21μCi ^{14}C(u)-glucose to a hen as a pure radiochemical. The 0.1m*l* of solution A has 28.5 nCi as raw activity here. Hence, 25m*l* of this solution should be injected to provide 7.1μCi pure activity.

要 約

炭水化物과 脂質代謝의 生動力學을 硏究하기 爲하여 正常産卵鷄에 注射하게 되는 均一標識^{14}C-포도당의 放射化學的 純度를 調査하였다. 炭素-14의 放射能을 測定하기 爲하여 液體 신치레숀 카운터를 利用하였으며 溶液中의 蛋白質을 沈澱시키기 爲하여 水酸化바륨과 黃酸亞鉛을 使用하였다. 注射溶液中의 포도당 濃度를 Hultman의 方法에 依하여 測定한 結果 0.912 mg/m*l* 이었으며 同 注射溶液의 比放射能은 포도당 1mg 當 31.3 nCi 이었다.

注射溶液에서 純粹 포도당을 分離하기 爲하여 五醋酸化포도당을 合成하였다. 分離한 純粹 포도당의 比放射能은 포도당 1mg 當 28.5 nCi 인 것으로 나타났다. 따라서 上記 注射溶液의 放射化學的 純度는 82.7%인 것으로 밝혀 졌다.

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