# THE METABOLISM OF GLUCOSE IN NORMAL AND THIAMINE DEFICIENT PIGEONS*

## I. THE CONVERSION OF GLUCOSE INTO GLYCOGEN, FATTY ACIDS AND RESPIRATORY $CO_2$

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SUMMARY

The *in vivo* incorporation of $^{14}C$ from uniformly labelled glucose into glycogen, fatty acids, and respiratory $CO_2$ has been studied with groups of pigeons that had received a fat-free diet, with and without thiamine, for 10 days. These three metabolic pathways were all found to be partially blocked in the thiamine deficient animals. The decreased incorporation was not caused by a slower absorption of the glucose from the digestive tract.

These observations can be attributed to a diminished activity of pyruvic dehydrogenase and possibly α-ketoglutaric dehydrogenase *in vivo*.

A remarkably large part of the label could not be accounted for, 2 h after the absorption of radioactive glucose was practically complete. This point is briefly discussed.

## INTRODUCTION

For several years investigations have been conducted in this laboratory concerning the changes occurring in animal tissues in the course of the development of a thiamine deficiency (*e.g.*, ref.[1-7]). These investigations have mainly dealt with the disappearance of thiamine pyrophosphate (TPP)—the metabolically active form of the vitamin—from the tissues on various diets, and with the decrease of anaerobic decarboxylative activity and *in vitro* reactivation of some enzymes having TPP as a prosthetic group, namely, pyruvic dehydrogenase and α-ketoglutaric dehydrogenase.

As expts. of this kind only throw some light on limited aspects of the complex changes taking place in the living animal during the development of the deficiency, it seemed interesting also to approach the problem by examining the effect of withholding the vitamin upon some major metabolic pathways in the intact organism. This question was not entirely new, but the expts. carried out in this field, *e.g.* by

* This work forms part of investigations on the metabolism and physiological function of thiamine, carried out by H. G. K. WESTENBRINK and collaborators.

Abbreviations used: TPP = thiamine pyrophosphate; TCA = trichloroacetic acid; r.s.a. = relative specific activity; ATP = adenosine triphosphate.

*References p. 484.*

McHENRY and collaborators[8–11], and GRUBER[2,3], were contradictory and—as will be shown in the discussion—inconclusive. In view of the role of thiamine as a constituent of several enzymes involved in the breakdown of carbohydrates, we have studied some transformations of glucose in the living organism, both under normal and thiamine deficient conditions. To this end glucose uniformly labelled with $^{14}C$ was administered orally to pigeons. These animals were chosen because they are very suitable for forced feeding and can be brought fairly rapidly to a state of advanced thiamine deficiency. The present report is concerned with the incorporation of radio carbon from glucose into glycogen, fatty acids, and respiratory $CO_2$ of normal and thiamine deficient animals.

## MATERIALS AND METHODS

### *Feeding procedure*

The daily dose of food, which was introduced into the gizzard through a glass tube after mixing with water, had the following composition: glucose 18 g, casein 2 g, salt mixture[12] 0.3 g, vitamin mixture[3] (without thiamine) 0.09 g. The thiamine content[13] of the glucose and casein was 0.03 and 0.25 $\mu$g/g, respectively; from the above diet the pigeons thus derived 1.0 $\mu$g of thiamine daily. All birds, both those that were rendered deficient and the controls (here called "normal"), were given this diet. In addition, the normal birds received 100 $\mu$g of thiamine daily. All had free access to water.

Before the first synthetic meal was given, the pigeons were divided into groups in such a way, that the average weight was the same within each group.

The liver glycogen contents of pigeons were found to vary to a considerable extent, even when all the birds were in the same nutritional state. *E.g.*, in a group of 13 pigeons that had been fed the daily dose of "synthetic" food for 3 days at 5.30 p.m., and killed early the next morning, these contents ranged from 0.10 to 1.25 g.

The most appropriate treatment for reducing these fluctuations in liver glycogen turned out to be the following. All pigeons received the synthetic diet (with or without thiamine) once a day for 7 days. The daily dose was then divided into 3 equal portions, which were administered around 9.00 a.m., 1.00 p.m., and 5.00 p.m., respectively, for 3 days. In the morning of the eleventh day the birds received the radioactive glucose. Uniformly labelled $^{14}C$-glucose (1 mC/10 to 15 mg: Radiochemical Centre, Amersham) was dissolved in 5 ml of distilled water and stored at $-15°$. The required quantity was added shortly before use to "carrier" glucose solution. Each pigeon received 10 ml of this solution, containing 4 g of glucose, by stomach tube. When 4 g of glucose were thus given after this pretreatment, liver glycogen ranged from 0.14 to 0.41 g after 4 h. Control expts. and occasional checks rarely showed more than 240 mg of the test dose of glucose in the digestive tract after 1 h, indicating the rapid absorption of at least 94 % of the glucose within this time.

### *Isolation and determination of metabolites*

*Glucose:* To check the absorption of glucose from the digestive tract, the isolated tract was extracted with boiling water. Proteins were precipitated with trichloroacetic acid (TCA) and removed by centrifugation. Glucose was determined in the remaining extract with anthrone, by the procedure of MORRIS, as modified by TREVELYAN AND HARRISON[14].

*References p. 484.*

*Glycogen and fatty acids*: After death by decapitation, the liver and the rest of the pigeon ("carcass") were dissolved separately by prolonged heating in conc. KOH, to ensure complete hydrolysis of fats. From these crude solutions both glycogen and fatty acids were isolated and purified.

Glycogen was purified by the method outlined in Fig. 1. Fairly early in the procedure total glycogen was assayed by the anthrone method[14]. Glycogen was again determined by the same method in the purified sample which was used for estimation of radioactivity.

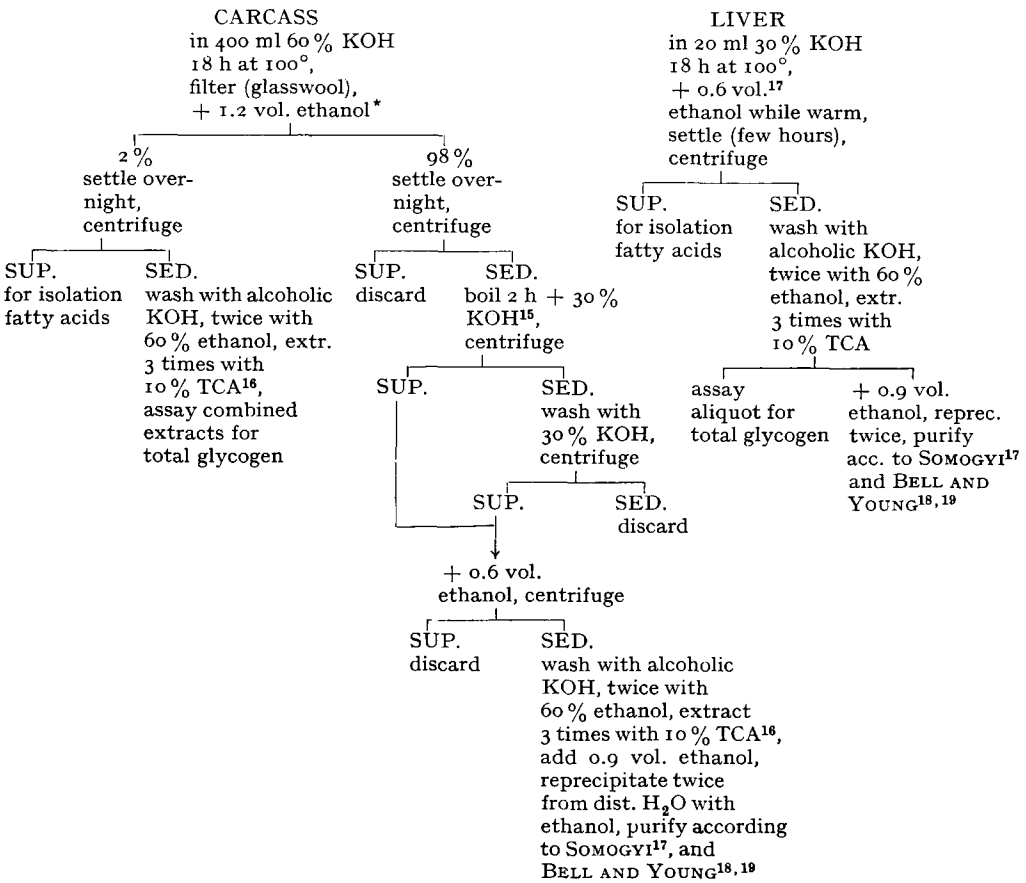


Fig. 1. Isolation and purification of glycogen.

In the preliminary expts. outlined in the previous section a rapid method of only moderate accuracy was required for the determination of liver glycogen. A known fraction of the liver was homogenized in 5 vol. of cold 5 % TCA in a Potter-Elvehjem homogenizer for 3 min. After centrifuging the sediment was again extracted twice with 5 vol. of cold 5 % TCA. The supernatant liquids were combined and assayed for glycogen with the anthrone reagent.

Fatty acids were isolated from the supernatant liquid remaining after ethanol

* Unless indicated otherwise, the ethanol used is 96 %.

*References p. 484.*

precipitation of glycogen in the crude extract, by the usual petroleum ether procedure[20,21]. The amount was determined by weighing the dry residue of an aliquot of the final petroleum ether solution[20].

*Respiratory $CO_2$:* An apparatus (Fig. 2) was constructed to enable simultaneous, separate, and continuous absorption of respiratory $CO_2$ from 6 pigeons. It was designed so that (a) the flow rate of the air leaving the apparatus could be controlled and measured for each individual, and (b) the expired $CO_2$ was absorbed almost instantly after expiration, *i.e.* the "dead space" in which the pigeon was breathing was reduced as much as possible.

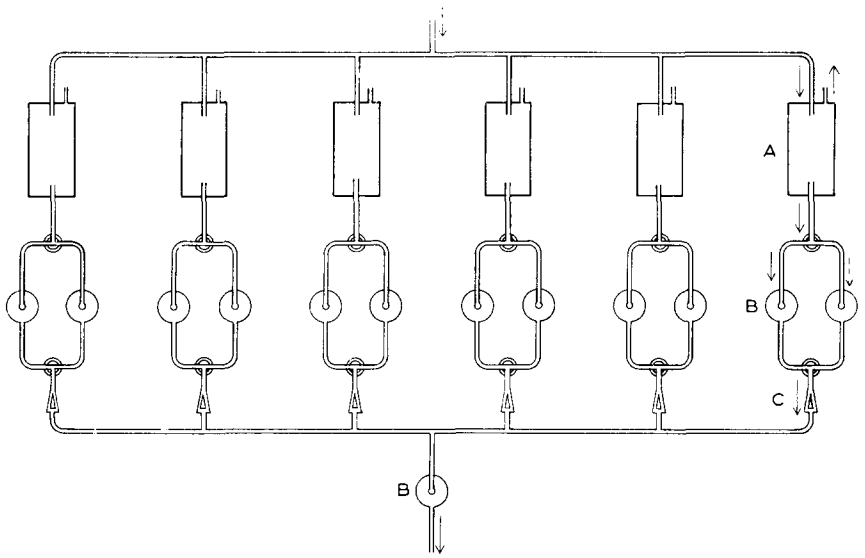


Fig. 2. Apparatus for the absorption of respiratory $CO_2$. A = box with pigeon; B = absorber with NaOH; C = flow meter.

Each pigeon was immobilized by strapping the wings to the body with adhesive tape and laid in a wooden box covered with a close-fitting perspex lid. A glass "diving helmet" with air inlet and outlet was slipped over the bird's head and fastened around the neck with adhesive tape (Fig. 3). $CO_2$-free air entered the box at slight overpressure, passed through the helmet inlet and was drawn out through the helmet outlet, which protruded through a wall of the box, into an absorber containing carbonate-free

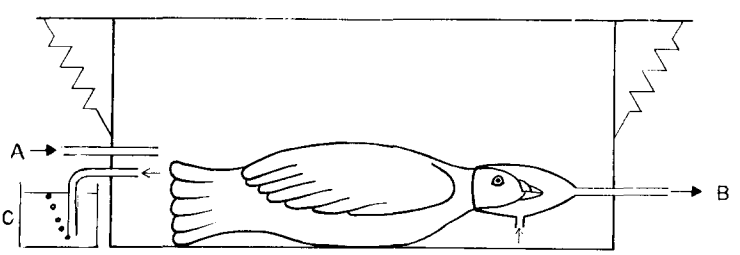


Fig. 3. Pigeon in box for absorption of respiratory $CO_2$. $CO_2$-free air enters the box at A at a rate of over 300 ml/min. and is drawn off through B together with the expired $CO_2$ at a rate of 300 ml/min. The excess air escapes through bubbler C (see text).

*References p. 484.*

10 % NaOH, through a fritted disperser (ref.[22], p. 83). At the chosen flow rate of 300 ml/min more than 99.5 % of the $CO_2$ expired was absorbed in this single absorber. A second absorber was placed parallel to the first and connected to the line in such a way that the expired air could be switched to this vessel by turning two 3-way stopcocks. This permitted subdivision of the total time, during which the $CO_2$ was collected continuously, into smaller periods. At the end of the line a large absorber was installed to act as a safety trap for all six individual absorbers. The excess air entering the box could escape from it through a bubbler, which also served as a check on the maintenance of overpressure. This overpressure prevented outside air containing $CO_2$ from leaking into the box, which was not always hermetically sealed.

The carbonate was precipitated as $BaCO_3$ from an aliquot of the absorbing alkali by adding $NH_4Cl$ (ref.[22], p. 85) and $BaCl_2$, and determined by weighing the dried $BaCO_3$.

*Estimation of radioactivity and percentage incorporation of $^{14}C$*

*Glucose:* An aliquot of the radioactive glucose test solution given to the pigeons was wet-ashed in the apparatus developed by ENTENMAN *et al.*[23], with the combustion mixture proposed by VAN SLYKE *et al.*[24]. The $CO_2$ formed was absorbed in carbonate-free 10 % NaOH, precipitated as $BaCO_3$, and filtered in the apparatus described by HENRIQUES *et al.*[25]. Sufficient glucose was ashed to mount the $BaCO_3$ in an "infinitely thick" layer for counting.

*Glycogen:* Glycogen was counted as such on aluminum counting trays. A homogeneously mounted layer was obtained as follows: 25 mg of glycogen were dissolved in 1 ml of distilled water, and 1.5 ml of 96 % ethanol added. (Pure glycogen is not precipitated by ethanol unless a trace of electrolyte is present.) The strongly opalescent, sometimes opaque, emulsion obtained remained stable for at least several weeks at room temp. The emulsion was added dropwise to an aluminum dish covered with lens paper (18 mm in diameter) placed at 30 cm distance under a 250 W infrared lamp on a cast iron base. The lens tissue was not essential, but helped in determining the moment when the sample was dry. Prolonged heating resulted in loosening of the sample, together with the lens paper, from the dish.

For the calculation of the percentage incorporation of $^{14}C$ it was necessary to compare the activities of the glycogen isolated and the glucose fed. As glucose was counted as $BaCO_3$, conversion factors had to be determined so that the observed counts/min of a sample of glycogen of known weight could be converted to the equiv. counting rate of this glycogen counted as an infinitely thick sample of $BaCO_3$. Part of a preparation of highly purified radioactive glycogen was converted into an infinitely thick sample of $BaCO_3$. The remainder was used for direct mounting of samples of various (measured) weights. Conversion factors $f$ were calculated from the equation:

$$f = \frac{\text{corrected counts from infinitely thick } BaCO_3 \text{ sample}}{\text{corrected counts from glycogen sample}}$$

As both numerator and denominator are proportional to the specific activity of the glycogen preparation, $f$ is independent of this activity. By plotting $f$ against mg of glycogen, a curve was obtained which could be used for the computation of the "$BaCO_3$ activities" of all glycogen preparations. These activities served to calculate

*References p. 484.*

the percentage of the $^{14}C$, administered as glucose, incorporated into the glycogen preparations.

*Fatty acids:* Fatty acids were mounted directly from petroleum ether solution as described by ENTENMAN *et al.*[23]. The percentage of $^{14}C$ incorporated from glucose into the fatty acids was calculated after conversion of "counts from fatty acids" to "counts from $BaCO_3$" with the aid of an empirical curve established as described for glycogen. This method is somewhat simpler than that used by ENTENMAN *et al.*

*Respiratory $CO_2$:* $CO_2$ was precipitated and mounted as $BaCO_3$ from the solution in NaOH, as described for glucose. Counting rates were determined on infinitely thick samples of $BaCO_3$, and the percentage incorporation of $^{14}C$ calculated by direct comparison with the counting rate of the $BaCO_3$ obtained from the test dose of glucose.

All preparations were counted under an end-window Geiger counter, mounted on aluminum planchets. The diameter of all samples was 18 mm. All preparations were counted for a period of time such that the statistical counting error (a few percent) became negligible in comparison to the biological variation. For the carcass fatty acids, which were of very low activity, a somewhat larger statistical error was considered permissible in view of the large difference between samples from normal and deficient pigeons.

## EXPERIMENTS AND RESULTS

### *Incorporation of $^{14}C$ into glycogen and fatty acids*

*In a first experiment* 38 normal and 40 thiamine deficient pigeons were each given 4 g of glucose, containing 8 $\mu$C of uniformly labelled glucose. Groups of 9 or 10 normal and groups of 10 deficient animals were decapitated 1, 2, 4, and 8 h after the administration of the labelled glucose. The livers were rapidly excised and saponified. Glycogen and fatty acids were isolated from the crude solution, the total amount determined, and the radioactivity measured after purification.

The results are assembled in Tables I and II. The values pertaining to 3 deficient pigeons which lost a considerable amount of fluid from the gizzard when decapitated are not included in the tables. Several livers contained too little glycogen to permit isolation in a purified form. For the calculation of the significance of the differences between the groups, the Wilcoxon test was used. The difference found was considered to be statistically significant when $P \leqq 0.050$. The P-values are given in all cases where the difference is significant, and, for comparison, in some other cases.

It can be seen from Table I that: (1) Incorporation of $^{14}C$ from glucose into glycogen was decidedly slower in the deficient animals. (2) The liver glycogen content seemed to decrease more slowly in the deficient animals in the period of 4 to 8 h after administration of radioactive glucose (the total glycogen of group N8 was significantly lower than that of group N4, whereas between groups D8 and D4 this difference was not quite significant). This could indicate that mobilization of glycogen during this period is impeded in deficient pigeons; but it is also possible that the maximum glycogen content was attained later than in the normal animals, and that the true rates of decrease were not significantly different. Such a shift could escape observation with the times chosen in this expt. (3) From 4 to 8 h after ingestion of radioactive glucose, the percentage of $^{14}C$ incorporated in glycogen decreased in both normal and deficient birds. This decrease, which was more marked in the normal group, would

*References p. 484.*

TABLE I

INCORPORATION OF $^{14}C$ FROM UNIFORMLY LABELLED GLUCOSE INTO LIVER GLYCOGEN

N = normal (control), D = thiamine deficient; group numbers refer to time (hours) between administration of $^{14}C$ and death. Stated are mean values and standard deviations of the means (standard errors), with numbers of pigeons in brackets. R.s.a. = relative specific activity = $= \frac{\text{specific activity of sample}}{\text{specific activity of glucose}} \times 100$. P-values according to WILCOXON.

| | *Group* | *Total glycogen (g)* | *R.s.a.* | *% Incorporated* |
|---|---|---|---|---|
| | N1 | 0.21 ± 0.02 (9) | 14 ± 3 (9) | 0.73 ± 0.18 (9) |
| | N2 | 0.24 ± 0.01 (10) | 34 ± 4 (10) | 2.3 ± 0.3 (10) |
| | N4 | 0.27 ± 0.03 (9) | 26 ± 5 (9) | 2.0 ± 0.5 (9) |
| | N8 | 0.10 ± 0.04 (10) | 6.8 ± 1.6 (6) | 0.21 ± 0.06 (6) |
| | D1 | 0.15 ± 0.03 (8) | 11 ± 3 (9) | 0.40 ± 0.11 (7) |
| | D2 | 0.28 ± 0.07 (7) | 17 ± 5 (9) | 1.1 ± 0.6 (7) |
| | D4 | 0.33 ± 0.07 (7) | 32 ± 5 (8) | 3.2 ± 1.0 (6) |
| | D8 | 0.13 ± 0.03 (7) | 18 ± 4 (8) | 0.97 ± 0.34 (6) |
| P-values | D1 vs. N1 | 0.08 | | |
| | D2 vs. N2 | | 0.010 | 0.021 |
| | N4 vs. D4 | | 0.40 | 0.39 |
| | N8 vs. D8 | 0.19 | 0.016 | 0.012 |
| | N1 vs. N2 | | 0.000 | 0.002 |
| | N8 vs. N4 | 0.001 | 0.006 | 0.000 |
| | D1 vs. D2 | 0.11 | 0.4 | 0.6 |
| | D2 vs. D4 | | 0.027 | 0.051 |
| | D8 vs. D4 | 0.053 | 0.050 | 0.041 |

TABLE II

INCORPORATION OF $^{14}C$ FROM UNIFORMLY LABELLED GLUCOSE INTO FATTY ACIDS OF THE LIVER

Same expt. as Table I. For further details see that Table.

| | *Group* | *No. of pigeons* | *Total fatty acids (g)* | *R.s.a.* | *% Incorporated* |
|---|---|---|---|---|---|
| | N1 | 9 | 0.23 ± 0.01 | 6.7 ± 0.2 | 0.72 ± 0.03 |
| | N2 | 10 | 0.24 ± 0.01 | 13 ± 1 | 1.4 ± 0.1 |
| | N4 | 9 | 0.21 ± 0.02 | 20 ± 1 | 1.9 ± 0.2 |
| | N8 | 10 | 0.24 ± 0.02 | 9.6 ± 0.9 | 1.1 ± 0.2 |
| | D1 | 10 | 0.20 ± 0.01 | 3.7 ± 0.7 | 0.36 ± 0.07 |
| | D2 | 9 | 0.27 ± 0.02 | 5.4 ± 1.5 | 0.64 ± 0.17 |
| | D4 | 9 | 0.21 ± 0.01 | 15 ± 2 | 1.5 ± 0.2 |
| | D8 | 9 | 0.25 ± 0.01 | 9.7 ± 0.8 | 1.1 ± 0.1 |
| P-values | D1 vs. N1 | | | 0.000 | 0.003 |
| | D2 vs. N2 | | | 0.004 | 0.005 |
| | D4 vs. N4 | | | 0.3 | 0.1 |
| | N1 vs. N2 | | | 0.002 | 0.000 |
| | N2 vs. N4 | | | 0.001 | 0.017 |
| | N8 vs. N4 | | | 0.000 | 0.008 |
| | D1 vs. D2 | | | 0.5 | 0.2 |
| | D2 vs. D4 | | | 0.005 | 0.009 |
| | D8 vs. D4 | | | 0.056 | 0.2 |

*References p. 484.*

be logically connected with the diminishment of glycogen. But the label disappeared from the glycogen more rapidly than the glycogen itself was consumed, resulting in a decrease in specific activity. This may be explained in terms of glucosidic exchange and metabolic inhomogeneity of the enormous glycogen molecule[26].

Table II shows that: (1) The rate of incorporation of $^{14}C$ from glucose into fatty acids was also markedly reduced in the deficient birds. (2) The percentage of $^{14}C$ incorporated and the specific activity of the fatty acids decreased more slowly in the deficient pigeons.

*In a second experiment* 9 normal and 10 deficient pigeons were each given 4 g of glucose, containing 50 $\mu$C of $^{14}C$*. More radioactive glucose was given this time because it was expected that specific activities would be lower in the carcass, which we now intended also to examine, than in the liver.

3 h after the administration of the labelled glucose the animals were decapitated. Glycogen and fatty acids from the separate livers and carcasses were isolated, the amount determined, and samples were assayed for radioactivity after purification. The results of the measurements are recorded in Tables III and IV.

TABLE III

INCORPORATION OF $^{14}C$ FROM UNIFORMLY LABELLED GLUCOSE INTO GLYCOGEN

N = normal, D = deficient, L = liver, C = carcass. Pigeons killed 3 h after administration of radioactive glucose. For further details see Table I and text.

| | *Group* | *No. of animals* | *Total glycogen (g)* | *R.s.a.* | *% Incorporated* |
|---|---|---|---|---|---|
| | NL | 8 | 0.41 ± 0.04 | 40 ± 6 | 4.4 ± 0.7 |
| | DL | 10 | 0.33 ± 0.03 | 47 ± 4 | 4.4 ± 0.6 |
| | NC | 9 | 1.6 ± 0.2 | 4.6 ± 0.4 | 2.2 ± 0.4 |
| | DC | 10 | 1.2 ± 0.1 | 3.1 ± 1.3 | 1.1 ± 0.4 |
| P-values | DC *vs.* NC | | 0.053 | 0.015 | 0.003 |

TABLE IV

INCORPORATION OF $^{14}C$ FROM UNIFORMLY LABELLED GLUCOSE INTO FATTY ACIDS

Same expt. as Table III. For further details see Tables I and III and text.

| | *Group* | *No. of animals* | *Total fatty acids (g)* | *R.s.a.* | *% Incorporated* |
|---|---|---|---|---|---|
| | NL | 8 | 0.27 ± 0.02 | 21 ± 2 | 2.5 ± 0.1 |
| | DL | 10 | 0.26 ± 0.03 | 11 ± 1 | 1.2 ± 0.1 |
| | NC | 9 | 53 ± 4 | 0.18 ± 0.02 | 4.3 ± 0.4 |
| | DC | 10 | 40 ± 4 | 0.09 ± 0.01 | 1.5 ± 0.2 |
| P-values | DL *vs.* NL | | | 0.000 | 0.000 |
| | DC *vs.* NC | | 0.053 | 0.001 | 0.000 |

* Although the animals had been distributed to form 2 groups of equal mean weight before the first forcibly fed meal, the average loss of weight up to the administration of the radioactive glucose appeared to be 14 and 43 g, respectively, for the normal and deficient pigeons. This difference was distinctly significant (P (Wilcoxon) = 0.006). It may be ascribed wholly or in part to vomiting[3,6] during the preparatory period, although severe vomiting was not observed. The radioactive glucose was never vomited.

*References p. 484.*

The figures obtained in this expt. for percentage incorporation of $^{14}C$ and relative specific activity (r.s.a.) in liver fatty acids agree well with those found in the previous expt. For liver glycogen there is at least a qualitative agreement; the r.s.a. and percentage of $^{14}C$ incorporated were higher in the normal than in the deficient pigeons after 2 h and (not significantly) higher in the deficient than in the normal birds after 4 h, whereas in this expt., after 3 h, they were equal for both groups. The percentage of $^{14}C$ now incorporated after 3 h was much higher than would be expected from the previous expt.; this may be due to seasonal differences (first expt. in September, second in January).

The incorporation of $^{14}C$ into carcass glycogen and carcass fatty acids was found to be markedly reduced in the deficient birds. The lower glycogen and fat contents of the deficient carcasses may again be ascribed, at least in part, to vomiting during the preparatory period.

A comparison of the specific activities reveals that the rate of incorporation of $^{14}C$ into both glycogen and fatty acids is very much higher in the liver than in the remainder of the body.

*Incorporation of $^{14}C$ into respiratory $CO_2$*

This expt., in which 9 normal and 9 thiamine deficient pigeons were examined, was performed in 3 runs. In each run three normal and three deficient pigeons each received 4 g of glucose, containing 8 $\mu$C of uniformly labelled glucose. Respiratory $CO_2$ was absorbed continuously for 8 h, divided into 15 periods of 20, 30, or 60 min each. Samples of the $CO_2$ expired in each period were assayed for radioactivity as infinitely thick layers of $BaCO_3$, and the amount determined by weighing the $BaCO_3$.

The combined results are shown in Figs. 4 and 5. In Fig. 4 the mean percentage incorporation of $^{14}C$/10 min in each collection period is given as the ordinate. The length of these periods is indicated on the abscissa. In all periods from 20 min up to 6 h the incorporation of $^{14}C$ was significantly lower in the deficient than in the normal birds (P-values ranging from 0.000 to 0.007). The same was true for the amount of $CO_2$ expired. Within each group, however, the total amount of $CO_2$ expired/min by all pigeons was about equal in nearly all periods (average over all periods: 12.0 mg $CO_2$/min/pigeon for the normal and 10.4 mg $CO_2$/min/pigeon for the deficient birds).

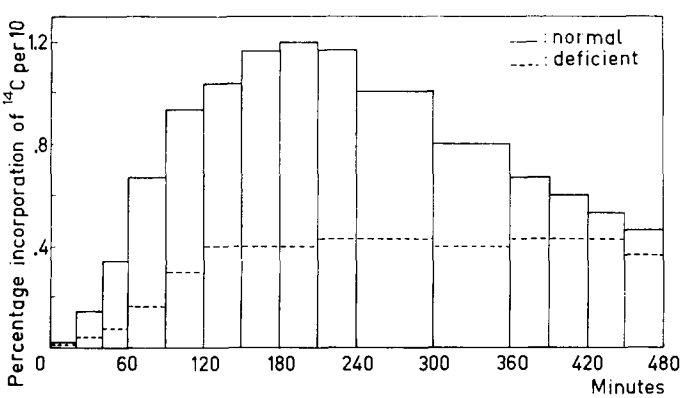


Fig. 4. Incorporation of $^{14}C$ into respiratory $CO_2$ during the collection periods. Ordinate: percentage incorporation of $^{14}C$/10 min. Abscissa: time, in minutes.

*References p. 484.*

Therefore the specific activities follow the same course as the percentage incorporation of $^{14}C$ and have not been depicted.

Fig. 5 shows the mean cumulative percentage incorporation of $^{14}C$ in $CO_2$ during the expt. for each group. After 8 h the total incorporation of $^{14}C$ and the total amount of $CO_2$ expired were also significantly different for the normal and the deficient groups (P-values 0.000 and 0.013, respectively).

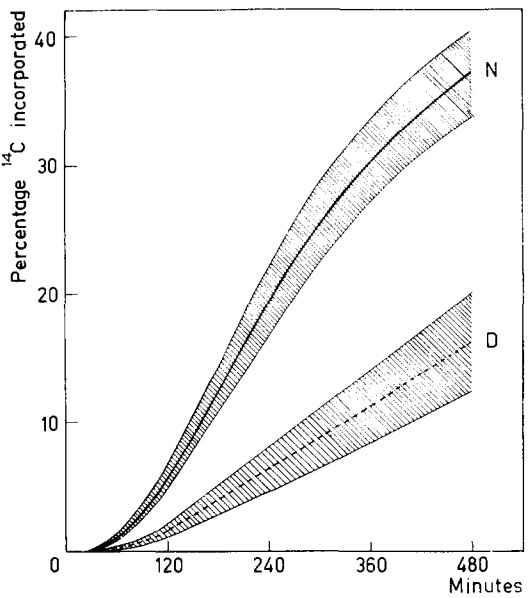


Fig. 5. Total incorporation of $^{14}C$ into respiratory $CO_2$. Ordinate: percentage $^{14}C$ incorporated. Abscissa: time, in minutes. N = normal, D = deficient. The standard error of the mean is indicated by the hatched area.

## DISCUSSION

The expts. reported above show that under conditions of thiamine deficiency the rate of incorporation by pigeons of $^{14}C$ from uniformly labelled glucose into the glycogen and fatty acids of the liver and of the remaining body ("carcass"), and into respiratory $CO_2$, is diminished. The rate at which the label disappears from liver glycogen and fatty acids, and from respiratory $CO_2$, is also slowed down in the deficient birds.

The retarded appearance and disappearance of $^{14}C$ in the fatty acids and respiratory $CO_2$ during thiamine deficiency was to be expected and can easily be explained. Because of a diminished decarboxylative activity of pyruvic dehydrogenase[7], the formation of acetyl-coenzyme A will be retarded, thus slowing down the conversion of glucose into fatty acids and the oxidation of glucose to $CO_2$. The oxidation of glucose and fatty acids may also be impeded by a possible diminished activity of another TPP-containing enzyme, α-ketoglutaric dehydrogenase. This is in accordance with early observations of SIMOLA[27], who demonstrated that the pyruvate content of blood and the α-ketoglutarate content of urine were enhanced in thiamine deficient rats.

The effect of thiamine deficiency in retarding the conversion of glucose into

*References p. 484.*

glycogen seems less obvious at first sight, for there is no enzyme containing TPP operating on the direct pathway from glucose to glycogen. However, a secondary effect of the deficiency may play a part. Diminished activity of pyruvic dehydrogenase (and α-ketoglutaric dehydrogenase) will slow down the rate of revolution of the citric acid cycle and thus less ATP, which is necessary for the synthesis of glycogen from glucose, will be formed. From our expts. the decrease in the rate of conversion of glucose into glycogen seems to be more pronounced in the carcass than in the liver. Presumably, most of the glucose residues incorporated into carcass glycogen have previously formed part of liver glycogen. To appear in carcass glycogen they would have to pass twice through the chain of reactions leading from glucose to glycogen, and thus would experience additional hindrance from diminished formation of ATP.

Ultimately, all the observations recorded above can be attributed to diminished activity of pyruvic dehydrogenase *in vivo* in pigeons which for 10 days have been fed a diet rich in carbohydrate, containing no fat and no thiamine.

For many years there has been controversy regarding the impairment of the synthesis of fat from carbohydrate in thiamine deficiency. McHENRY and his collaborators[8–11] concluded that impairment existed, but GRUBER[2,3] could not find a diminished fat synthesis.

As GRUBER justly remarks, McHENRY's observations may be explained by the fact that he did not apply forced feeding, for one of the first occurrences in the development of a thiamine deficiency is a loss of appetite. In one experiment[8] McHENRY applied paired feeding to rats, but as only mean values are given without indication as to the statistical significance of the (small) difference between the means, the evidence cannot be regarded as conclusive.

GRUBER conducted 2 expts. with forcibly fed pigeons. In the first he observed that animals retained their fat stores after 12 days on a fat- and thiamine-free diet. As the degradation as well as the synthesis of the fatty acids could be impaired in thiamine deficiency, no conclusion regarding the velocity of the synthesis of fat from carbohydrate could be drawn. In the second expt., two (small) groups of pigeons were first depleted of 80–90 % of their fat by prolonged feeding with very little mixed cereals, followed by 7 days on a minimum quantity of thiamine-free food. They were then given an abundant quantity of fat-free food, with and without thiamine respectively. After 6 days the amount of fat resynthesized was not significantly different in the 2 groups. However, we hesitate to regard this expt. as conclusive for the following reasons. Firstly, there were only 6 pigeons in each group, and their final body fat contents were scattered over a wide range: 5.8 to 26.4 g in the controls and 4.3 to 14.3 g in the deficient animals (normal average: 44 g). Secondly, the controls had been partially depleted of their thiamine in the preparatory period, so that it is doubtful if these could be regarded as "normal" pigeons, at least at the onset of the 6-day restoring period.

The investigation with labelled glucose reported in this paper does appear to provide direct evidence that when pigeons have been fed a well-defined fat-free diet with or without thiamine for 10 consecutive days, the conversion of carbohydrate into fat is retarded in the birds lacking thiamine.

It should be pointed out that although in our expts. at least 94 % of the glucose ingested was already absorbed from the gut within 1 h in nearly all cases, a surprisingly large portion of the $^{14}C$ was not recovered in the combined glycogen, fatty

*References p. 484.*

acids, and respiratory $CO_2$, 3 h after the administration of the labelled glucose (76 % and 88 % in normal and deficient pigeons, respectively). Thus, even in normal pigeons most of the glucose administered does not seem to be metabolized along what might have been considered the major metabolic pathways for glucose. A preliminary search for the missing $^{14}C$ showed that only very little was to be found in the excrements, the bones, and the proteins of liver and of breast muscle (*i. e.*, the largest portion of the skeletal muscle). When, however, a normal pigeon was homogenized and extracted with a mixture of ethanol and ether (3:1) 3 h after administration of 25 $\mu$C of $^{14}C$-glucose, approx. 40 % of the total radioactivity was recovered from the extract. Lipids extractable with petroleum ether accounted for only a few percent of this radioactivity, nearly all of which was present in the watery residue. The latter contained about 7 g of dry matter. The nature of the compound(s) isolated in this way is being investigated.

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