Estimation of the Glucuronic Acid Pathway Contribution to Glucose Metabolism in Adipose Tissue and the Effect of Growth Hormone*

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BERNARD R. LANDAU,† GLENN E. BARTSCH, AND HOLLIS R. WILLIAMS
From the Departments of Medicine and Biochemistry, and the Division of Biometry, Western Reserve University, Cleveland, Ohio 44106

SUMMARY
A definite contribution of the glucuronic acid pathway to total glucose metabolism by rat adipose tissue in vitro in the presence of growth hormone preparations has not been found. Randomization of 14C of glucose-5-14C into glycerol and glucose-1-14C and -2-14C into glycogen on incubation of rat epididymal tissue indicates that the pathway contributes at most only a few per cent to the over-all metabolism of glucose. Results of incubations with glucose-1-14C and -6-14C are in accord with metabolism solely via the pentose cycle, Embden-Meyerhof pathway, and Krebs cycle. Thus, essentially all of the 14C utilized on incubation of glucose-1-14C and -6-14C is isolated in products formed via these pathways. Nonequilibration of dihydroxyacetone phosphate with glyceraldehyde 3-phosphate occurs, and this results in a preferential oxidation to CO2 of carbon 6 as compared to carbon 1 via the Krebs cycle. This coupled with an increase in the fraction of carbon 3 of pyruvate converted to CO2 and a decreased pentose cycle contribution in the presence compared to the absence of the preparations can account for the enhanced yields of 14CO2 from glucose-6-14C as compared to -1-14C that have been reported on growth hormone addition.

Presentation of a growth hormone preparation to adipose tissue in vitro stimulates the oxidation to CO2 of carbon 6 of glucose relative to carbon 1, so that frequently yields of 14CO2 from glucose-6-14C are greater than from glucose-1-14C (1, 2). Winegrad et al. (1, 3) considered this response most likely to be due to a stimulation of glucose metabolism via the glucuronic acid pathway, a pathway by which carbon 6 of glucose, but not carbon 1, is oxidized to CO2. An alternative explanation, considered less probable, was that it was attributable to isotopic nonequilibration of dihydroxyacetone phosphate with glyceraldehyde 3-phosphate, a mechanism by which carbon 6 relative to carbon 1 of glucose would be preferentially oxidized to CO2 in the Krebs cycle. Isotopic nonequilibration has been shown in incubations of adipose tissue in vitro (2, 4–7).

Portions of this work were done during the tenure of an Established Investigatorship of the American Heart Association.

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750
Glucose-6-P, 5-14C → dihydroxyacetone-P + glyceraldehyde-3-P, 2-14C

The metabolism of a molecule of glucose-5-14C via the pentose cycle also yields a molecule of glyceraldehyde-3-P, 2-14C (9).

3 Glucose-6-P, 5-14C → 3 CO2 + 3 pentose-5-P, 4-14C
3 Pentose-5-P, 4-14C → 2 glucose-6-P, 5-14C + glyceraldehyde-3-P, 2-14C

Sum: glucose-6-P, 5-14C → 3 CO2 + glyceraldehyde-3-P, 2-14C

In contrast, the metabolism of a molecule of glucose-5-14C via the glucuronic acid pathway (10) yields pentose-5-P, 5-14C, and the latter’s subsequent metabolism via the pentose cycle yields a molecule of glyceraldehyde-3-P, 3-14C.

3 Glucose-6-P, 5-14C → 3 glucuronic acid-5-14C
3 Glucuronic acid-6-14C + 3 CO2 → 3 L-xylulose-1-14C
3 L-Xylulose-1-14C → 3 D-xylulose-5-14C
3 D-Xylulose-5-14C → 2 glucose-6-P, 6-14C + glyceraldehyde-3-P, 3-14C

Sum: glucose-6-P, 5-14C → 3 CO2 + glyceraldehyde-3-P, 3-14C + 2 glucose-6-P, 6-14C

Glucose-5-14C was incubated in vitro with rat epididymal adipose tissue in the presence of growth hormone and the glycogen formed, presumed to reflect the 14C distribution in glyceraldehyde-3-P, was isolated and degraded. An estimate of the glucuronic acid pathway contribution to over-all glucose metabolism was then made from the ratio of 14C incorporated into C-3:C-2 of glycerol.

From glucose-2-14C via the pentose cycle, glucose-6-P, 1, 2, 3, 4-14C is formed and via the glucuronic acid pathway, glucose-6-P, 2, 3, 4-14C (9, 10). Via the pentose cycle, carbon 1 of glucose-1-14C is evolved as 14CO2 while via the glucuronic acid pathway it is randomized into carbon atoms 2 and 3 of glucose-6-P. Glucose-2-14C and -1-14C were incubated with adipose tissue and glucose from glycogen, presumed to reflect the 14C distribution in glucose-6-P, isolated and degraded. Estimates of the glucuronic acid pathway contribution were then made from the relative incorporation of 14C into carbon atoms 1, 2, and 3 of the glucose from glycogen.

To achieve the second objective, the pentose cycle contribution to glucose metabolism was estimated from the randomization of carbon 2 of glucose 2-14C during its incorporation into glycogen and glycerol (11) and the extent of equilibration of triose phosphates from the incorporation of 14C of glucose-1-14C and -6-14C into glycerol and fatty acids (8). Parallel incubations in the absence of growth hormone were performed to establish a hormone effect.

Within the limits of these estimates, we conclude that the glucuronic acid pathway contribution to the total metabolism of glucose can be no more than a few per cent. Metabolism of glucose via the pentose cycle, Embden-Meyerhof pathway, and Krebs cycle, in the presence of nonequilibrium of triose phosphates, adequately explains the change in 14CO2 yields observed with growth hormone preparations.

**EXPERIMENTAL PROCEDURES**

**Materials and Incubations**—The procedure was similar to that previously described (6). Male albino rats of the Wistar strain, weighing 200 to 250 g and fed ad libitum on stock chow, were decapitated. Their epididymal fat pads were removed, trimmed, and distributed so that each of the incubation flasks in an experiment contained one segment from each of the rats killed. Segments with a total weight of 1.0 to 2.1 g, from 8 to 20 rats, were present in a flask. A serum bottle of 30-ml capacity served as the flask. It was closed by a rubber serum stopper from which a vial was suspended. The medium was 2 ml of Modified Krebs bicarbonate buffer (7). Glucose, present at a concentration of 2 mg per ml, was labeled with 1-14C, 2-14C, 5-14C, and 6-14C. Glucose-1-14C and -6-14C were purchased from New England Nuclear Corporation. They were purified to remove "carbonate-like" material (12). Glucose-2-14C was purchased from Nuclear Chicago Corporation. Glucose-5-14C was generously provided by Dr. Lars Ljungdahl. Its preparation and the confirmation of the position of the label by degradation have been reported (13). A bovine growth hormone preparation was added to the medium at a concentration of 1.0 mg per ml. The hormone, a gift of the Endocrinology Study Section of the National Institutes of Health (Lots NIH-GH-B-7 and B-8), was reported to assay at a potency of about 0.9 unit per mg. A human growth hormone preparation assaying at about twice the potency of the U.S.P. growth hormone reference preparation was a gift of Dr. Olof Pearson (14). It was added at a concentration of 0.2 mg per ml. After gassing with 95% O2 and 5% CO2 the medium had a pH of 7.3 to 7.5. Incubation was with shaking for 3 hours at 37°C. In each of four experiments, glucose-1-14C and -6-14C were incubated in the absence and presence of growth hormone. Parallel incubations were performed with glucose-2-14C and -5-14C in the presence of growth hormone. A control incubation was performed in which the flask and contents were identical with those with 14C except that only nonlabeled glucose was present.

**Isolation of Products**—Incubations with glucose-1-14C and -6-14C were terminated by injection through the stopper of 0.4 ml of 30% perchloric acid into the medium of each flask and 1 ml of hyamine into each vial. After shaking for an additional hour, each vial was removed and capped.

The medium was withdrawn with a Pasteur pipette to a graduated centrifuge tube. The flask and fat pads were rinsed with water and these washings were combined with the medium (aqueous phase). A negligible quantity of 14C was extracted with water and these washings were combined with the medium (aqueous phase). Glucose in the aqueous phase and rinses were added to the lipid phase. The aqueous phase was neutralized to phenolphthalein with potassium hydroxide. After cooling the potassium perchlorate precipitate was removed by centrifugation. The precipitate carried negligible 14C activity. Glucose utilization was determined by preparing glucosazones from the initial solution and the aqueous phase and assaying them for 14C. Glucose in the aqueous phase was also determined with glucose oxidase (15). An aliquot of the aqueous phase was acidified and extracted continuously with ether. Carrier lactate was added to the new
tralized extract which was then evaporated and the lactate was purified on a Celite column. The lactate was combusted and assayed for $^{14}C$, giving the quantity of $^{14}C$ incorporated into lactate. The aqueous and initial solutions were also chromatographed on Whatman No. 3MIM paper with the upper phase of butanol, water, methanol, and formic acid (820:820:80:1 by volume), and lactate, glycerol, and glucose areas were identified through guide samples. These areas were eluted and assayed for $^{14}C$ activity to give the percentage of the added $^{14}C$ incorporated into glycerol, lactate, and glucose. The incorporation into lactate by this procedure was in good agreement with the results obtained by the more complicated isolation and combustion of the lactate. Therefore, the former method was used in all but the first experiment. The determination of residual glucose by this chromatographic method was also in good agreement with that obtained with glucose oxidase or osazone formation. Chromatography was performed with a pyridine, ethyl acetate, acetic acid, water solution (5:5:1:3 by volume) (16), with a glucuronic acid guide sample, to estimate $^{14}C$ activity in the area corresponding to glucuronic acid. In addition, chromatography was done with a butanol, acetic acid, water solvent (4:1:5 by volume) (17) with xylose as a guide spot to estimate $^{14}C$ in compounds having the mobility of the common pentoses.

An aliquot of the lipid phase was washed with water and the $^{14}C$ in the water phase assayed. This water soluble $^{14}C$ material, considered within the tissue, represented less than 5% of the $^{14}C$ added to the incubation media. On chromatography, the $^{14}C$ was present in glucose and glycerol, with the former accounting for 80% or more of the activity. In the tables to follow, this $^{14}C$ activity in glucose has been included in the estimate of recovered glucose. Another aliquot of the lipid phase was evaporated and the fatty residue hydrolyzed for 12 to 24 hours in 70% ethanolic KOH at 70-80°C. The solution was evaporated, acidified, and extracted with heptane. The $^{14}C$ in the heptane was taken as incorporation into fatty acids and that in the aqueous layer as incorporation into glycerol after correcting for the $^{14}C$ present as glucose. In two experiments, the solution was extracted prior to acidification to measure the $^{14}C$ incorporated into non-saponifiable lipid. The activity in this fraction was less than 1% of the $^{14}C$ in the initial medium.

The tissue residue was decomposed in 30% KOH. The resulting solution was neutralized to phenophthalein, taken to volume, and an aliquot assayed for $^{14}C$ activity. This was taken to represent the $^{14}C$ in glycerogen and supporting tissue. The remaining solution was acidified with H$_2$SO$_4$ and heated at 100°C for 3 hours to hydrolyze the glycerogen. Carrier glucose was added and the glucosazone was made and assayed for $^{14}C$ in comparison with the osazone of the initial medium, giving the percentage of incorporation into glycerogen. Incorporation of $^{14}C$ into supporting tissue was obtained by difference.

In the incubations with glucose-2-$^{14}C$ and -5-$^{14}C$ the fat pad segments were removed after incubation and immersed in chloroform-methanol. Glycerol and glucose from glycogen were isolated and degraded as previously detailed (7). The specific activity of the glucose-5-$^{14}C$ (2000 dpm per mg) was too low to yield adequate incorporation into glycerogen for degradation and assay, so that with glucose-5-$^{14}C$ only glycerol was degraded. As a control glycerol-2-$^{14}C$ was degraded. Lactate isolated from the medium as described above was also degraded. In one experiment, glycerogen was isolated and degraded after incubation with glucose-1-$^{14}C$ and -6-$^{14}C$ in the presence of growth hormone. In a fifth experiment, glucose-1-$^{14}C$ and -6-$^{14}C$ were incubated in the presence of bovine and human growth hormone and in their absence. The yields of $^{14}CO_2$ established an effect for the hormone similar to that observed in the first four experiments. Glucose from glycogen, isolated from the tissues incubated with glucose-1-$^{14}C$ and hormone, was then degraded.

**Counting Procedures**—Glucosazones were plated on stainless steel planchets and assayed in a gas flow, thin window counter. Aliquots from the heptane, aqueous, and hyamine solutions were assayed in a dioxane solution (18) with a liquid scintillation spectrometer. Counting of the final solutions was done simultaneously with aliquots from the initial solutions so that the percentage of the $^{14}C$ added to the flask which was incorporated into the products could be calculated directly. Aliquots from the products of the control incubation were added to the initial samples and aliquots of the control initial samples to the product samples to achieve identical quenching (6). Channel ratios were employed to verify equal degrees of quenching.

$^{14}CO_2$ obtained from the individual carbon atoms of glucose and glycerol on degradation was usually evolved into ethanalamine which was then dissolved in toluene containing a scintillant (19) and assayed in the spectrometer. In a few instances where $^{14}C$ activity was very low, samples were assayed in the gas phase.

**RESULTS**

**Incorporation of $^{14}C$ from Glucose-1-$^{14}C$ and -6-$^{14}C$ into Products**

—Table I records tissue weights, the percentage of the added glucose utilized, and the recovery in the products of $^{14}C$ from the glucose-1-$^{14}C$ and -6-$^{14}C$.

The percentage of the utilized $^{14}C$ from glucose-1-$^{14}C$ oxidized to $^{14}CO_2$ was decreased by growth hormone while the percentage from glucose-6-$^{14}C$ was increased. The percentage of $^{14}C$ from both glucose-1-$^{14}C$ and -6-$^{14}C$ incorporated into glycerol was increased by growth hormone. Much more $^{14}C$ was recovered in glycerol in the aqueous phase. The percentage of the utilized carbon incorporated into fatty acids decreased. The preferential incorporation of carbon 6 of glucose relative to carbon 1 into fatty acids is clearly demonstrated. Except for the last experiment, less than 4% of the utilized $^{14}C$ was incorporated into lactate. The percentage of $^{14}C$ in lactate was greater from glucose-6-$^{14}C$ than -1-$^{14}C$. Incorporation into glycerogen and supporting tissue was a small percentage of utilized $^{14}C$ and appeared to be decreased by the addition of growth hormone.

The percentage of recovery of the utilized $^{14}C$ as measured by the summing of the percentage of the utilized $^{14}C$ found in $^{14}CO_2$, glycerol, fatty acids, lactate, glycerogen, and tissue residue was nearly 100%, except for Experiment 1 (last column in Table I). Almost all of the $^{14}C$ in the aqueous phase was accounted for in glucose, glycerol, and lactate, except in Experiment 1 where as much as 20% was not. By paper chromatography (16), $^{14}C$ was found in an area corresponding to the $R_6$ of glucuronic acid. However, the $^{14}C$ assayed at no more than 2% of the utilized $^{14}C$ and there was as much $^{14}C$ in the absence as in the presence of growth hormone. With butanol-acetic acid-water as solvent (17), no $^{14}C$ activity was detected in the area corresponding to the mobility of xylose and ribose.
Incorporation of $^{14}$C of glucose-1-14C and -6-14C into products on incubation with rat adipose tissue in absence and presence of growth hormone

Glucose was at a concentration of 2 mg per ml. Incubation was for 3 hours at 37°. Isolation of the various fractions and their assay is described in the text. In Experiments 1 and 2, a bovine growth hormone preparation was added at a concentration of 1.0 mg per ml. In Experiments 3 and 4, a human growth hormone preparation was added at a concentration of 0.2 mg per ml.

### Table I

**Glucose utilization and distribution of $^{14}$C in various fractions.**

| Experiment | Growth hormone | Tissue weight | Percentage of $^{14}$C of utilized glucose recovered in | CO$_2$ | Glycerol | Fatty acids | Lactate | Glycogen | Supporting tissue | $^{14}$C recovery%
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* Utilized glucose as determined from the difference between the $^{14}$C added and recovered in glucose; assayed as glucosazones.
† Sum of the percentage of the $^{14}$C of the utilized glucose recovered in CO$_2$, glycerol, fatty acids, lactate, glycogen, and supporting tissue.

### Table II

Distributions of $^{14}$C in glycerol and glycogen on incubation of glucose-1-14C and -6-14C with adipose tissue in presence of growth hormone.

Incubations were under the same conditions and run parallel to the incubations with growth hormone of Table I. Relative distributions are recorded with carbon 2 = 100. Recovery obtained by totaling the $^{14}$C found in each carbon, as a percentage of the $^{14}$C in the product on combustion, is recorded to serve as a measure of the adequacy of the degradation procedure.

<table>
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<tr>
<th>Experiment</th>
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<th>Glycol* glucose-2-14C as substrate</th>
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* Glycerol-2-14C, purchased from Orlando Chemical Company, when degraded gave a distribution of 3.3, 100, 1.9 (recovery 94%).
† In this experiment distribution in the glucose unit of glycogen on incubation of glucose-2-14C in the absence of growth hormone was 12.9, 100, 6.5, 1.6, 13.3, 1.2 (90% recovery).

Randomization of $^{14}$C from $^{14}$C-labeled Glucoses into Glycerol, Glycerol, and Lactate—In Table II, the distribution of $^{14}$C on degradation of glycerol and glucose from glycogen after incubation of glucose-2-14C or -5-14C with growth hormone is recorded. With glucose-5-14C, $^{14}$C appeared in carbon 1 as well as 3 of glycogen, but there was less than $\frac{3}{2}$ as much $^{14}$C activity in carbon 3 as 2. When a commercial preparation of glycerol-2-14C was degraded (see asterisk footnote of Table II), similar amounts of $^{14}$C were found in carbon atoms 1 and 3. Carbon 2 of glucose-2-14C was randomized into the other carbon atoms of glycerol and of the glucose from glycogen and the distributions in the glycerol and glucose were similar. The distribution in glycogen was similar to that observed in one experiment in the absence of growth hormone (dagger footnote of Table II). Lactates isolated from
hormone was $C_1 = 6.5$, $C_2 = 16.4$, $C_3 = 100$, with a recovery of 98%. The distribution in lactate in the absence of growth hormone was $C_1 = 1.6$, $C_2 = 3.3$, $C_3 = 100$, with a recovery of 98%.

Glucose-6-$^{14}$C

Glucose-5-$^{14}$C

Glucose-1-$^{14}$C

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* In this experiment glycerol was also degraded. The relative distribution was $C_1 = 1.6$, $C_2 = 3.3$, $C_3 = 100$, with a recovery of 98%. The distribution in lactate in the absence of growth hormone was $C_1 = 6.5$, $C_2 = 16.4$, $C_3 = 100$, with a recovery of 98%.

**TABLE IV**

**Distribution of $^{14}$C in glucose unit of glycogen on incubation of glucose-1-$^{14}$C and 5-$^{14}$C in presence of growth hormone**

The $^{14}$C of the position in the glucose with the highest $^{14}$C concentration has been set equal to 100 and incorporation in the remaining carbon atoms is relative to this carbon. Samples are from Experiment 4 of Table I and Experiment 5.

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<td>4.3*</td>
<td>0</td>
<td>9.7</td>
</tr>
<tr>
<td>Experiment 5†</td>
<td>100</td>
<td>1.3</td>
<td>1.1</td>
<td>12.6</td>
</tr>
<tr>
<td>Glucose-6-$^{14}$C</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Experiment 4</td>
<td>0.4</td>
<td>1.0*</td>
<td>2.7</td>
<td>100</td>
</tr>
</tbody>
</table>

* Sum of the activity in carbons 2 and 3.
† Bovine growth hormone.
‡ Human growth hormone.

The aqueous phase in experiments with glucose-5-$^{14}$C and 6-$^{14}$C were degraded (Table III). There was extensive randomization of carbon 5 of glucose into carbon atoms 1 and 3 and of carbon 6 of glucose into carbon atoms 1 and 2 of lactate. A much smaller randomization of carbon 6 occurred during its incorporation into glycerol than lactate (footnote to Table III). In Experiment 4, glucose from glycogen isolated after incubation with glucose-1-$^{14}$C and 5-$^{14}$C in the presence of growth hormone was degraded (Table IV). $^{14}$C of glucose-6-$^{14}$C was localized to carbon 6 of the glucose from glycogen, but about 10% of the $^{14}$C of glucose-1-$^{14}$C was randomized into carbon 6. About 4% as much activity was in carbon atoms 2 and 3 as in carbon 1 of the glucose from glycogen with about twice as much $^{14}$C in carbon atom 3 as 2 (Experiment 5).

**DISCUSSION**

**Estimation of Glucuronic Acid Pathway Contribution**

The contribution of the glucuronic acid pathway to overall glucose metabolism can be estimated from the randomization of the specifically $^{14}$C-labeled glucoses. The estimates are independent of the extent of equilibration of the triose phosphate except with glucose-5-$^{14}$C.

Glucose-5-$^{14}$C—The contribution of the glucuronic acid pathway to glucose metabolism is reflected by the incorporation of $^{14}$C of glucose-5-$^{14}$C into carbon 3 relative to carbon 2 of glycerol. The results of Table II would indicate that this amounted to no more than 3% of the total glucose utilized (2.7/(100 + 2.7)) in the experiment in which the greatest randomization was observed.† The degradation of glycerol upon which this estimate is based is not completely specific (21). Thus, in a degradation running parallel to the degradations of these experiments, about 3% of the activity in carbon 2 of glycerol-2-$^{14}$C appeared in carbon 3 (asterisk footnote of Table II) and Bjornholm and Wood (21) found 1.9% of the activity of a glycerol-2-$^{14}$C sample appeared in carbon 3. Also extensive randomization of carbon atoms 5 and 6 of glucose occurred in the Krebs cycle, as evidenced by the distribution of $^{14}$C in lactate on incubation with glucose-5-$^{14}$C and 6-$^{14}$C (Table III). Possibly $^{14}$C in carbon atoms 1 and 3 of glycerol with glucose-5-$^{14}$C as substrate was incorporated after metabolism via the Krebs cycle. Randomization of $^{14}$C in the Krebs cycle and then incorporation into glycerol has been shown to occur on incubation of specifically labeled pyruvate with mouse adipose tissue (22). A small quantity of $^{14}$C from glucose-6-$^{14}$C in the absence of added hormone has been found in carbon atoms 1 and 2 of glycerol with *Aerobacter aerogenes* for degradation (7), and this is again shown in one of the experiments of the present series (footnote to Table III). Therefore, some or all of the small percentage of $^{14}$C appearing in carbon 3 of glycerol with glucose-5-$^{14}$C as substrate is probably due to conditions other than metabolism via the glucuronic acid pathway.

Glucose-2-$^{14}$C and -1-$^{14}$C—The randomization of $^{14}$C from glucose-2-$^{14}$C into carbon atoms 1 and 2, and of glucose from glycogen can also be used to estimate the glucuronic acid pathway contribution. As shown in the “Appendix” (Equation 21), in the presence of the glucuronic acid pathway randomization via the pentose cycle should fulfill the relationship previously derived for a system in which the pathway was absent (11, 23), $x_1/x_2 = 2PC/(1 + 2PC)$. $X_1/x_2$ is the relative specific activity in C-1: C-2 of glucose-6-P, PC is the fraction of total metabolism via the cycle and the equilibration of fructose-6-P with glucose-6-P is assumed to be complete ($E_2 = 1$). In the absence of the glucuronic acid pathway, $x_1/x_2 = (x_1/x_2)/(2 - x_1/x_2)$ should also hold (11), but in its presence, $x_1/x_2$ should be greater than $x_1/x_2/(2 - x_1/x_2)$. Based on the assumption that the glucuronic acid pathway is absent, $x_1/x_2$ should be, with the mean value for $x_1$ in this calculation, the rate of the transaldolase exchange reaction (20) is assumed to be zero (in the appendix expressions are derived which do not require this assumption). The incorporation of $^{14}$C into carbon 3 of glycerol is assumed to be solely via the glucuronic acid pathway, where, for each molecule of glucose-5-$^{14}$C metabolized in the pathway, a molecule of 5-keto-2-deoxyglucose is formed, while for each molecule metabolized via the Embden-Meyerhof pathway or pentose cycle a molecule of 2-deoxyglucose-6-P is formed (see introduction). Since this hexose-6-P will be metabolized to triose-P via the pentose cycle and Embden-Meyerhof pathway, $^{14}$C will also be incorporated into carbon 3 of glycerol via these pathways. Therefore, in the calculation some of the $^{14}$C in carbon 3 attributed to the glucuronic acid pathway is from these other pathways. This results in an overestimation of the glucuronic acid pathway contribution.
of paper chromatography are evidence for the nonaccumulation of carbon 3 on degradation (12, 25). If it is assumed to be due entirely to a glucuronic acid pathway contribution, the contribution could account for about 2% of total glucose metabolism. This is true even though $E_{1,2}$ is finite (see "Appendix" for the details of these calculations). Randomization of $^{14}$C from glucose-1-$^{14}$C can also be used to estimate the contribution of the glucuronic acid pathway contribution since carbon 1 of glucose is randomized into carbon atoms 2 and 3 of glucose-6-P by this pathway, but not by the pentose cycle. From the data of Table IV a contribution of about 3.5% is estimated (see "Appendix"). Reversal via the pentose cycle will also make this an overestimation. Furthermore, the small incorporation of $^{14}$C into carbon atoms 2 and 3 approaches the limits of the degradation procedure.

Assumptions—The above estimates of the glucuronic acid pathway contribution depend upon the assumption that glucose utilization can proceed only via the Embden-Meyerhof pathway, pentose cycle, glucuronic acid pathway, and Krebs cycle. The recoveries in Table I would support this assumption since, except for a small quantity of $^{14}$C incorporated into glycerogen, essentially all of the $^{14}$C utilized appeared in products of these pathways. The estimates also require that the glucuronic pathway be complete, that is that the carbon atoms of glucose entering the pathway, other than carbon 6, are metabolized via pentose 5-phosphate. If intermediates of the glucuronic acid pathway accumulated, randomization of $^{14}$C of the $^{14}$C-labeled glucose into glycerol and glycojen would not reflect the quantity of glucose entering the pathway. The completely complete recoveries of $^{14}$C in the products isolated (Table I) and the results of paper chromatography are evidence for the nonaccumulation of intermediates. Therefore, the increase in the oxidation of carbon 6 of glucose to CO$_2$ in the presence of growth hormone cannot be attributed in more than a small measure to metabolism via the glucuronic acid pathway.

Pathways followed by Glucose Carbon in Presence of Growth Hormone

The increase in the oxidation of carbon 6 by growth hormone, as well as the decrease in the oxidation of carbon 1, relative to glucose utilization is in accord with metabolism solely via the Embden-Meyerhof pathway and the pentose and Krebs cycles in the presence of incomplete equilibration of the triose phosphates. The quantity of glucose carbon converted to the various products required to produce the yields of $^{14}$C given in Table I and the randomizations patterns in Tables II to IV can be estimated. This is done by the use of the model of Katz, Landau, and Bartsch (6). The transaldolase reactions (50), which are omitted, do not affect the results if pathways which do not form triose phosphate, i.e. non-triose-P pathways, are absent. As already noted, in adipose tissue the major non-triose-P pathway is glycogen formation and its contribution is too small to affect the estimations. Equilibration of the hexose 6-phosphates is assumed to be sufficiently extensive to be considered complete. This is the case for adipose tissue unstimulated by hormone and the similar patterns of $^{14}$C randomization in carbon atoms 1, 2, and 3, and carbon atoms 6, 5, and 4 of glucose from glycerogen, and carbon atoms 3, 2, and 1 of glycogen (Table II) indicate this is also so when growth hormone is active (7, 20).

The estimation procedure parallels that already used for data on the effect of growth hormone on adipose tissue from rats fasted and then refed (6). The yields from glucose-1-$^{14}$C and -6-$^{14}$C in Table I have been converted to specific yields and the ratio of the specific yields from glucose-1-$^{14}$C to -6-$^{14}$C for fatty acids, glycerol, and lactate calculated (Table V). Note the similar ratios for fatty acids and lactate and the increased difference between the glycerol and fatty acids ratios produced by growth hormone. From the data in Table V, the pentose cycle contribution, the relative rate of triose-P isomerization in both directions, the fraction of glucose metabolized to glycerol and pyruvate, and the specific activities of dihydroxyacetone-P and glyceraldehyde 3-phosphate relative to that of glucose have been estimated. The pentose cycle contribution calculated from the specific yields of $^{14}$CO$_2$ averaged 19% (range 18 to 20%) in the absence of hormone and 8% (range 2 to 14%) with hormone. Under similar experimental conditions with no hormone present, estimates based on the randomization of $^{14}$C of glucose-2-$^{14}$C into glycogen averaged only 9% (7), and this is in agreement with the value obtained (8).

### Table V

<table>
<thead>
<tr>
<th>Growth hormone</th>
<th>$^{14}$C label</th>
<th>Specific yields*</th>
<th>Ratio of specific yields</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{14}$C</td>
<td>Fatty acids</td>
<td>Glycerol</td>
</tr>
<tr>
<td>Absent</td>
<td>1</td>
<td>24 (10-31)</td>
<td>19 (11-24)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>46 (22-90)</td>
<td>26 (18-36)</td>
</tr>
<tr>
<td>Present</td>
<td>1</td>
<td>15 (7-21)</td>
<td>42 (37-52)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>28 (9-45)</td>
<td>36 (30-47)</td>
</tr>
</tbody>
</table>

* Values are the averages with the ranges in parentheses for the four experiments of Table I. The percentage of specific yields of a product equals $((^{14}$C yield in the product/1$^{14}$C in the glucose utilized)) $\times 100$. Since almost all of the $^{14}$C of the $x_1/x_2$ of Table II, 0.122/1.878 $= 0.065$, rather than the observed value 0.081. The greater observed than predicted value may be due to reversal of the pentose cycle (23, 24). It may also be due, at least in part, to contamination of carbon atom 3 by 2 during degradation, since as much as 1% of the activity of glucose-2-$^{14}$C is found in carbon 3 on degradation (12, 25). If it is assumed to be due entirely to a glucuronic acid pathway contribution, the contribution could account for about 2% of total glucose metabolism.

The estimation procedure parallels that already used for data on the effect of growth hormone on adipose tissue from rats fasted and then refed (6). The yields from glucose-1-$^{14}$C and -6-$^{14}$C in Table I have been converted to specific yields and the ratio of the specific yields from glucose-1-$^{14}$C to -6-$^{14}$C for fatty acids, glycerol, and lactate calculated (Table V). Note the similar ratios for fatty acids and lactate and the increased difference between the glycerol and fatty acids ratios produced by growth hormone. From the data in Table V, the pentose cycle contribution, the relative rate of triose-P isomerization in both directions, the fraction of glucose metabolized to glycerol and pyruvate, and the specific activities of dihydroxyacetone-P and glyceraldehyde 3-P relative to that of glucose have been estimated. The pentose cycle contribution calculated from the specific yields of $^{14}$CO$_2$ averaged 19% (range 18 to 20%) in the absence of hormone and 8% (range 2 to 14%) with hormone. Under similar experimental conditions with no hormone present, estimates based on the randomization of $^{14}$C of glucose-2-$^{14}$C into glycogen averaged only 9% (7), and this is in agreement with the value obtained (8).
the distribution observed in Experiment 4 (dagger footnote of Table II). The rates of triose-P isomerization relative to glucose utilization were less with hormone than without (relative rates averaging 1.4 compared to 2.5). However, as previously noted (6), increased utilization with growth hormone makes the absolute rates similar. The specific activity of glyceraldehyde-3-P is calculated to be 1.17 times the specific activity of dihydroxyacetone-P relative to that of glucose-6-14C with growth hormone present (averaged 1.18 compared to 0.84). With glucose-1-14C, the activity of dihydroxyacetone-P is higher than that of glyceraldehyde-3-P (averaged 1.00 compared to 0.72). The differences in specific activity are less when growth hormone is absent (relative values 1.17 compared to 1.00 and 0.74 compared to 0.60).

These estimates require that the triose phosphates once formed do not recombine to hexose-6-P in significant quantity. Evidence that this latter requirement is fulfilled for adipose tissue in the absence of hormone addition has been detailed (7). The failure of 14C from glucose-6-14C to appear in carbon 1 of glucose from glycogen (Table IV) is evidence for fulfillment in the presence of growth hormone. The incorporation of 14C of glyceraldehyde-3-P into carbon 6 of glucose from glycogen can be attributed to the transaldolase reactions (7, 20).

With these calculations, the absolute quantities of the carbon of the glucose utilized which were converted to fatty acids, glycerol and lactate carbon, and CO2 during the incubations have been estimated (Table VI) (6). Similar quantities of carbon were converted to fatty acids in the presence of growth hormone, as in its absence, despite the greater utilization of glucose in the presence of hormone. Growth hormone increased glyceraldehyde carbon incorporation to glucose utilization (43.1% of glucose carbon utilized compared to 27.1%), while the quantity of glucose carbon to lactate was a similar percentage of the glucose carbon utilized. The quantity of CO2 formed in the pentose cycle was similar with or without hormone although the percentage of the glucose carbon converted to CO2 was less with growth hormone (4% compared to 10%). CO2 formation by decarboxylation of pyruvate paralleled glucose utilization, but with growth hormone a greater percentage of glucose carbon utilized was oxidized to CO2 in the Krebs cycle. In the estimates of CO2 formed in the Krebs cycle, the specific yield of 14CO2 from glucose-6-14C has been adjusted for the quantity of 14CO2 formed during the decarboxylation of pyruvate. This has been done by assigning the distribution of 14C in pyruvate to be the same as in lactate (Table III) so that approximately one-thirteenth of the 14C of glyceraldehyde-3-P is assumed to have been randomized to the carboxyl carbon of pyruvate. The quantity of TPNH formed via the pentose cycle could provide only one-third to two-thirds the hydrogen required for reduction of acetyl-CoA to the two-carbon unit of fatty acids. A similar result has been obtained under different conditions and with other hormones (6). Growth hormone also decreased the pentose cycle contribution in the experiments with fasted-refed rats (6). The percentage of carbon converted to lactate is less in the present study than those previously reported experiments (6).

**Other Evidence for Incomplete Equilibration of Triose Phosphates and Small Glucuronic Acid Pathway Contribution in Presence of Growth Hormone**

The isotopic nonequilibrium of triose-P in the presence of growth hormone, shown in these experiments, is in accord with the observations of Winegrad et al. (1). Thus, while incorporation in glycerol was not measured in their experiments, about twice as much 14C from glucose-6-14C as -1-14C was incorporated into fatty acids (Table VII of Reference 1). Since the 14C in their petroleum ether-extractable material was probably almost completely in triglyceride, the equal incorporation of carbon atoms 1 and 6 into this fraction would support a greater incorporation of carbon 1 relative to carbon 6 into glycerol. The measurements of glucose uptake and incorporation of carbon atoms 1 and 6 of glucose into CO2, glyceride-glycerol, fatty acids, and glycogen were in good agreement with those of LeBœuf and Cuhill (2) done under similar conditions. Their conclusions that growth hormone preparations decreased the pentose cycle contribution and increased the Embden-Meyerhof contribution to glucose metabolism and oxidation in the Krebs cycle were based on a calculation in which recycling was not considered.

Other data of Winegrad et al. (3), while not accompanied by measurements of glucose and glucuronic acid utilization and probably dependent on the concentrations of substrate selected, suggest that in the absence of added hormone the glucuronic acid pathway makes a relatively small contribution, at most, to over-all glucose metabolism in adipose tissue from fed rats. Thus, their yield of 14CO2 on incubation with glucuronic acid-6-14C was only 0.075 µmole per mg of tissue nitrogen while with
glucose-6-14C under similar conditions it was 0.24 amole per mg of tissue nitrogen. Every molecule of glucose-6-14C metabolized via the glucuronic acid pathway yields a molecule of 14CO2. All of carbon 6 of glucose metabolized via the Embden-Meyerhof pathway and pentose cycle appears in triose-P, but only a small fraction of carbon 3 of this triose-P is oxidized to CO2 (Table VI). Therefore, much more glucose-6-14C would have to be metabolized via the Embden-Meyerhof pathway and pentose cycle than glucuronic acid pathway to produce the same yields of 14CO2.

Other hormones showing lipolytic properties in adipose tissue also stimulate the oxidation of carbon 6 relative to carbon 1 and under these conditions, nonequilibration of triose-P occurs (2, 6, 24, 26). Lynn, MacLeod, and Brown (5), in their balance studies with adrenocorticotropic hormone, with a phosphate buffer, concluded that the glucuronic acid pathway makes at most a negligible contribution to over-all glucose metabolism, since 90% or more of the 14C utilized was converted to products other than intermediates of the pathway. They did not eliminate, as we have now done, metabolism occurring via the pathway to pentose phosphate and then subsequently via the pentose cycle.

Adequacy of Growth Hormone Preparations

The conditions of the present experiments were designed to approximate those of Winegrad et al. in their original studies (1). Large concentrations of the growth hormone preparations have been used. As stated by Winegrad and Shaw (8) the changes in metabolism observed on addition of the growth hormone preparations may be due to contaminants. Jungas and Ball (27) have considered thyrotropic hormone to be a possible contaminant causing the effects seen with growth hormone preparations. In a single experiment, under conditions similar to those employed with the growth hormone preparations, the effect of thryotropic hormone on the metabolism of glucose-l-i%, -2-14C, -5-14C, and -6-14C in adipose tissue were studied. A pattern of response similar to that seen with the growth hormone preparations was observed in that there was an increase in the oxidation of carbon 6 of glucose relative to carbon 1, an increase in the percentage of incorporation of 14C into glyceraldehyde-3-P, and a decrease in the percentage of incorporation into fatty acids, and a decrease in the pentose cycle as measured by the randomization of carbon 2 of glucose-2-14C into glycerol. Thus, the effects observed with the growth hormone preparations may not be due to growth hormone.

APPENDIX

Expressions are derived for estimating the contribution of the glucuronic acid pathway to glucose metabolism in the intact cell with the use of a model in which non-triose-P-forming pathways, the pentose and Krebs cycles, and the Embden-Meyerhof pathway are also active. These expressions are applied to the data for adipose tissue just presented. Estimates from the extent of randomization of 14C from glucose-2-14C or -1-14C and -6-14C in adipose tissue are utilized.

Fig. 1. Model of glucose metabolism. The model is identical with that presented in an accompanying paper (20) except that rate $V_i$ is introduced. This represents the rate of conversion of glucose-6-P carbon atoms to CO2 and pentose-5-P via the glucuronic acid pathway. As in the pentose cycle, 1/4 of the carbon atoms entering the pathway are oxidized to CO2. The remainder are metabolized to glyceric acid-3-P and converted to fructose-6-P. The fraction of hexose metabolized via the pathway is then $\frac{1}{4} \frac{V_i}{V_0}$.

In a single experiment, under conditions similar to those employed with the growth hormone preparations, the effect of thyrotropic hormone on the metabolism of glucose-2-14C, -5-i%, and -6-14C in adipose tissue were studied. A pattern of response similar to that seen with the growth hormone preparations was observed in that there was an increase in the oxidation of carbon 6 of glucose relative to carbon 1, an increase in the percentage of incorporation of 14C into glyceraldehyde-3-P, and a decrease in the percentage of incorporation into fatty acids, and a decrease in the pentose cycle as measured by the randomization of carbon 2 of glucose-2-14C into glycerol. Thus, the effects observed with the growth hormone preparations may not be due to growth hormone.

3B. R. Landau and J. Katz, unpublished observations.

4 In the model the glucuronic acid pathway is depicted as proceeding from glucose-6-phosphate (10). If glucose is converted to glucuronic acid without glucose 6-phosphate as an intermediate, the glucuronic acid pathway contribution to over-all glucose utilization would be overestimated.
four pools are assumed to remain unchanged and hence the following relationships hold.

\[
\begin{align*}
V_0 + V_{-\alpha} &= V_a + V_1 + V_4 + V_7 \\
V_a + \frac{1}{2} (V_1 + V_4) &= V_{-\alpha} + V_2 \\
\frac{1}{2} V_2 + V_{-\gamma} &= V_1 + V_4 \\
\frac{1}{2} V_4 + V_{-\gamma} &= V_1 + V_4 \\
\frac{1}{2} V_7 + V_{-\gamma} &= V_1 + V_4
\end{align*}
\]

The specific activities of glucose-6-P, fructose-6-P, dihydroxyacetone-P, and glyceraldehyde-3-P are denoted by \(z, y, u,\) and \(z,\) respectively, and those of the individual carbon atoms of these molecules are indicated by adding a subscript corresponding to the number of the carbon atom. For example, \(z_6\) denotes the specific activity of carbon 6 of glucose-6-P, and \(z_2\) denotes that of carbon 2 of glyceraldehyde-3-P. The specific activity of glucose as substrate is designated by \(G\) with a subscript corresponding to the position of the carbon bearing the label.

Glucose-3-\(^{14}\)C as Substrate—Equations 1 to 18 express the changes in \(^{14}\)C in each of the four pools.

\[
\begin{align*}
\frac{dM_1}{dt} &= V_{-\alpha} y_1 - (V_1 + V_2 + V_4 + V_7) z_1 \\
\frac{dM_2}{dt} &= V_4 G + V_{-\alpha} y_2 - (V_1 + V_2 + V_4 + V_7) z_2 \\
\frac{dM_3}{dt} &= V_{-\alpha} y_3 - (V_1 + V_2 + V_4 + V_7) x_1 \\
\frac{dM_4}{dt} &= V_{-\alpha} y_4 - (V_1 + V_2 + V_4 + V_7) x_2 \\
\frac{dM_5}{dt} &= V_{-\alpha} y_5 - (V_1 + V_2 + V_4 + V_7) x_3 \\
\frac{dM_6}{dt} &= V_{-\alpha} y_6 - (V_1 + V_2 + V_4 + V_7) x_4 \\
\frac{dM_7}{dt} &= V_{-\alpha} y_7 - (V_1 + V_2 + V_4 + V_7) x_5 \\
\frac{dM_8}{dt} &= V_{-\alpha} y_8 - (V_1 + V_2 + V_4 + V_7) x_6 \\
\frac{dM_9}{dt} &= V_{-\alpha} y_9 - (V_1 + V_2 + V_4 + V_7) x_7 \\
\frac{dM_{10}}{dt} &= V_{-\alpha} y_{10} - (V_1 + V_2 + V_4 + V_7) y_{10}
\end{align*}
\]

For isotopic steady state conditions these equations are equated to 0. Eliminating \(y_1\) from Equations 1 and 7 and replacing \(V_{-\alpha}\) by \((V_1 + V_2 - V_4 - V_7)\) and \(V_4\) by \((V_1 - 1/3 V_1 - 1/3 V_4 - V_3)\)

\[
\begin{align*}
\frac{x_2}{x_0} &= \frac{2/3V_1}{V_6 + 2/3V_1 + (V_0 - 1/3 V_1 - 1/3 V_4 - V_3) V_6/V_7} \\
\frac{x_5}{x_0} &= \frac{2 PC}{1 + 2 PC + (1-PC-GA-NTP)/E_{-h}}
\end{align*}
\]

When equilibration of the hexose 6-phosphates is complete \((E_{-h} \to \infty),\) Equation 20 simplifies to

\[
\frac{x_1}{x_0} = \frac{2 PC}{1 + 2 PC + (1-PC-GA-NTP)/E_{-h}}
\]

This is the result previously derived for a model in which no provision was made for the presence of the glucuronic acid pathway (11). Eliminating \(y_1\) from Equations 1 and 7 and \(y_3\) from Equations 3 and 9, the quotient of the two resulting equations yields, following rearrangement of terms, an expression for the relative contribution of the glucuronic acid pathway to that of the pentose cycle for any value of \(E_{-h}\) \((E_{-h} \neq 0),\) NTP \((NTP \neq 1),\) PC \((PC \neq 0),\) and GA \((GA \neq 1).\)

\[
\frac{GA}{2x_5/x_1 - 1 - x_3/x_2} = PC
\]

If the equilibration of the hexose 6-phosphates is assumed to be complete or nearly so, Equation 21 yields an estimate of PC and its substitution in Equation 22 gives an estimate for GA.

Following algebraic manipulations with Equations 1 to 18, an expression for estimating \(E_{-h}\) for the case of \(GA \neq 0\) is obtained.

\[
E_{-h} + 1 = \frac{(x_5/x_1 - x_3/x_2)}{(1 - x_3/x_2) \left[ x_2/x_1 - (x_3/x_4 \frac{x_2/x_3 - x_5/x_7}{x_2/x_5 - x_5/x_7}) \right]}
\]

Equations 20, 22, and 23 allow estimates of PC, GA, and \(E_{-h}.\) The estimates are independent of the relative rate of the transaldolase exchange reaction, \(E_{\alpha} \tau,\) where \(E_{\alpha} \tau = V_{\alpha} V_{\alpha}^{-1}\) and of \(V_{\alpha}/V_0.\) Of the three equations, only Equation 20 is a function of NTP. The results of their application to the data of Table II, assuming NTP = 0, are given in the first three columns.
of Table VII. If $E_{-h}$ is assumed to approach $x_3$ rather than being finite, the remaining values in the table are changed little except in Experiment 4 where $E_{-h}$ is negative. With $E_{-h} \to \infty$ for this experiment, $PC = 0.066$ and $GA = 0.028$.

After algebraic manipulations of Equations 1, 6, 12, 15, and 18, an upper bound on the estimate of $Ex_T$ is

$$Ex_T < \frac{1 + GA}{(1 - PC - GA) \times z_2/z_1 - (2 - PC + 2GA)}$$

(24)

$$- 1/2 (PC + GA)$$

The inequality given by Equation 24 is valid only if the denominator of the first term is positive. In its use to obtain an upper bound on $Ex_T$, an estimate of $V_{el}/V_t$ or its upper bound must be available. The estimates of $GA$ and $1^{14}C$ can be those with Equations 20, 22, and 23. Expressions for estimating the upper bound on $Ex_T$ are given for each experiment as well as the average value are recorded.

$GA$ is deleted from Equation 2. These two modified equations and Equations 3 to 18 represent the changes of $1^{14}C$ in each of the four pools when glucose-1-$^{14}C$ is substrate. Equations similar to Equations 19 to 23 can be derived.

Glucose-1-$^{14}C$ and -6-$^{14}C$ as Substrates—$V_{el}$ is added to Equation 1 and $V_{el}$ is deleted from Equation 2. These two modified equations and Equations 3 to 18 represent the changes of $1^{14}C$ in each of the four pools when glucose-1-$^{14}C$ is substrate. Expressions for estimating the upper bound on $Ex_T$ are given for each experiment as well as the average value are recorded.

Table VII

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$E_{-h}$</th>
<th>PC</th>
<th>GA</th>
<th>$V_{el}/V_t$</th>
<th>$Ex_T$</th>
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<tbody>
<tr>
<td>1</td>
<td>0.6</td>
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<td>0.020</td>
<td>0.81</td>
<td>0.49</td>
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<td>2.7</td>
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<td>0.009</td>
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<td>3</td>
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<td>0.54</td>
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</table>

Under the assumption of complete equilibration of the hexose 6-phosphates, the application of Equation 29 to the results of Experiment 56 of Table IV yields $GA = 0.035$ and to those of Experiment 57 $GA = 0.036$.

Following algebraic manipulations of the modified Equation 2 and Equations 3 to 18, an expression for estimating $E_{-h}$ when $GA \neq 0$ can be obtained and is

$$E_{-h} + 1 = \frac{\left(\frac{V_h}{V_t}\right) - 1}{\left(\frac{V_h}{V_t}\right) - 1 + \frac{1}{2} \left(\frac{V_h}{V_t}\right) - 1}$$

(30)

The four equations denoting the changes of $1^{14}C$ in each of the four pools when glucose-6-$^{14}C$ is substrate are given by Equations 31 to 34.

When equilibration of the hexose 6-phosphates is complete ($E_{-h} \to \infty$), Equation 26 reduces to

$$\frac{z_2}{z_1} = \frac{2PC}{1 + 2PC}$$

(27)

Eliminating $y_6$ from the modified equations, 2 and 8, and $y_6$ from Equations 3 and 9, the quotient of the two resulting equations yields, following rearrangement of terms, an expression for the relative contribution of the gluconic acid pathway to that of the pentose cycle for any value of $E_{-h} (E_{-h} \neq 0)$, NTP (NTP $\neq 1$), PC ($PC \neq 0$), and $GA$ ($GA \neq 1$).

$$GA = \frac{2x_2 - x_3(x_2 + x_3)}{x_2(x_2 + x_3) - 2x_2x_6}$$

(28)

If the equilibration of the hexose 6-phosphates is assumed to be complete or nearly so, Equation 27 yields an estimate of PC and its substitution in Equation 28 yields

$$V_{el}/V_t \leq \frac{1}{\gamma(1 - 3GA - NTP) G_6/G_1 - GA}$$

(35)

Application of Equation 35 has been presented in Table VII
with the ratio, $\gamma$, of the specific yields of fatty acids given in Table V. The estimates of $V_{sl}/V_{s}$ are similar with the specific yields of lactates. Although the estimate of $V_{sl}/V_{s}$ may have little intrinsic interest, it is required to obtain the bounds on $E_{x_{T}}$. The latter is required for estimating the glucuronic acid pathway contribution from data obtained when using glucose-5-\textsuperscript{14}C.

Glucose-5-\textsuperscript{14}C as Substrate—Equations 36 to 43 represent the changes in $\mu$C in each of the four pools.

$$\frac{dM_{x_{3}}}{dt} = V_{x_{3}} + V_{s} - (V_{s} + V_{l} + V_{x_{4}} + V_{f}) x_{3}$$

$$\frac{dM_{x_{4}}}{dt} = V_{x_{4}} + V_{s} - (V_{s} + V_{l} + V_{x_{3}} + V_{f}) x_{4}$$

$$\frac{dM_{y_{5}}}{dt} = (V_{s} + V_{l} + V_{x_{3}} + V_{x_{4}} + V_{f}) y_{5}$$

$$\frac{dM_{y_{6}}}{dt} = (V_{s} + V_{l} + V_{x_{3}} + V_{x_{4}} + V_{f}) y_{6}$$

$$\frac{dM_{z_{2}}}{dt} = V_{x_{2}} - (V_{s} + V_{l} + V_{x_{1}} + V_{f}) z_{2}$$

$$\frac{dM_{z_{3}}}{dt} = V_{x_{3}} - (V_{s} + V_{l} + V_{x_{2}} + V_{f}) z_{3}$$

$$\frac{dM_{z_{4}}}{dt} = V_{x_{4}} - (V_{s} + V_{l} + V_{x_{3}} + V_{f}) z_{4}$$

$$\frac{dM_{z_{5}}}{dt} = V_{x_{5}} - (V_{s} + V_{l} + V_{x_{4}} + V_{f}) z_{5}$$

$$\frac{dM_{z_{6}}}{dt} = V_{x_{6}} - (V_{s} + V_{l} + V_{x_{5}} + V_{f}) z_{6}$$

For isotopic steady state conditions, these 8 equations are set equal to 0. Equation 42 can be expressed in terms of $x_{5}, x_{4},$ and $z_{5}$ by solving Equation 38 for $y_{5}$, Equation 40 for $z_{4}$ and substituting these expressions in Equation 42. Similarly, Equation 43 can be expressed in terms of $x_{6}, x_{5},$ and $z_{6}$ by solving Equation 39 for $y_{6}$, Equation 41 for $z_{5}$ and substituting these expressions in Equation 43. Denoting $GA = \frac{1}{2} V_{s}/V_{n}$, $PC = \frac{1}{2} V_{s}/V_{o}$, and $E_{x_{T}} = V_{x_{T}}/V_{o}$ and noting that $PC \leq 1$, the inequality obtained from these expressions follows

$$GA < \left(\frac{u_{5}}{u_{2}} - \frac{u_{6}}{u_{4}}\right)(1 + E_{x_{T}})$$

Therefore, if the ratio of activity in C-3:C-2 in dihydroxyacetone phosphate or its derivative, glycerol, is known, an estimate can be made of the upper bound on the contribution of the glucuronic acid pathway. No assumptions of the contribution of the pentose cycle and of the non-triose-P pathways and the rates of isomerizations of the hexose $6P$-phosphates and of the triose phosphates are required.

When the inequality given by Equation 44 is applied to the data obtained with glucose-5-\textsuperscript{14}C (Table II with $E_{x_{A}}$ and $E_{x_{T}}$ as given in Table VII), GA is found to be no greater than 0.040, 0.010, and 0.000 (average 0.017) for Experiments 1, 2, and 4, respectively. If $E_{x_{A}}$ is assumed to approach $\infty$ the respective values for GA are unchanged. The contribution of glucuronic acid pathway to total glucose metabolism is therefore estimated at no greater than 1.7%.

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REFERENCES

Estimation of the Glucuronic Acid Pathway Contribution to Glucose Metabolism in Adipose Tissue and the Effect of Growth Hormone
Bernard R. Landau, Glenn E. Bartsch and Hollis R. Williams


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