Carbohydrate Metabolism in the Leukocytes

I. THE PATHWAY OF TWO- AND THREE-CARBON COMPOUNDS IN THE RABBIT POLYMORPHONUCLEAR LEUKOCYTE*

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One of the unique characteristics of polymorphonuclear leukocytes from normal subjects is their high rate of aerobic and anaerobic glycolysis with accumulation of lactic acid (1, 2). Intermediates and enzymes of the Embden-Meyerhof scheme have been isolated and identified (3-5) and Beck (6) has calculated the $V_{\text{max}}$ of a number of these enzymes. The results indicate the hexokinase to be the chief rate-limiting enzyme in human normal, chronic lymphocytic, and chronic myelocytic leukocyte homogenates. There are indications for an intact Krebs cycle in the human polymorphonuclear leukocyte (2, 7), even though the ratio of respiration to glycolysis has been found to be exceedingly low (8). In addition, the operation of the phosphogluconate oxidation pathway has been demonstrated by Coxon and Robinson (9) in white cells isolated from the peripheral blood of cats, rabbits, and dogs. Beck (10) in a further study has isolated and characterized certain enzymes of this oxidative pathway and has estimated about 3% of catabolized glucose to traverse this route in normal human leukocytes.

The present study attempts to assess the role of the various carbohydrate pathways in the overall metabolism of the white blood cell. $^{14}C$-labeled acetates, pyruvates, lactate, and glycerol have been incubated in vitro with rabbit polymorphonuclear leukocytes. The glycogen of the cells and the lactate of the medium were isolated, purified and the $^{14}C$ distribution pattern determined by degradation. A preliminary report of this work has been presented (11).

EXPERIMENTAL

Materials

Leukocytes—Male rabbits weighing 3.5 to 4.5 kg were given intraperitoneal injections of 200 ml of 7.2% sterile caseinate solution. Sixteen hours later, 500 ml of pH 7.4 isotonic phosphate buffer were injected into the peritoneal cavity. The exudate (97 ± 1% polymorphonuclear leukocytes and 3 ± 1% lymphocytes) was removed through a 15 gauge needle and collected in 250-ml siliconized centrifuge cups. After light centrifugation, the cells were washed with phosphate buffer, recentrifuged and suspended in Hank’s medium buffered at pH 7.4. Cell counts were obtained with the use of an AO Spencer Hemacytometer.

Serum—Two weeks before the administration of the caseinate solution, blood was obtained by cardiac puncture from the rabbit donating the leukocytes. After debribration, the cellular elements and the fibrin clot were removed by centrifugation in the cold and the pH of the serum adjusted to 7.4 with 1N HCl.

Radioactive Materials—Sodium acetate-1- and -2-Cl$^4$ and zinc lactate-2-C$^4$ were obtained from Volk Radiochemical Company. Sodium pyruvate-1-, 2-, and -3-C$^4$ were purchased from Nuclear-Chicago Corporation. Glycerol-1(3)-C$^4$ (m-glycerol-1-C$^4$) was a product of Research Specialties.

Methods

Incubations—Four series of experiments were carried out in siliconized Erlenmeyer flasks equipped with single side arms. The main compartment contained $2.0 \times 10^8$ polymorphonuclear leukocytes suspended in 10 ml of the donor rabbit’s serum and 5 ml of Hank’s buffer containing 0.22 mmole of nonlabeled glucose. $^{14}C$ substrates were each dissolved in 1 ml of Hank’s buffer and placed in the side arms. The vessels were fitted with serum caps and flushed with a gas mixture of 95% O$_2$-5% CO$_2$. The contents of the side arms were tipped into the main compartments of the vessels and the mixtures were incubated at 37° with shaking. After 4 hours of incubation, 2 ml of 0.2 N H$\text{SO}_4$ were added, and the cells were centrifuged and washed with Hank’s buffer in the cold (0-4°). The medium was saved for lactic acid isolation.

Isolation and Degradation of Glucose from Glycogen—Glycogen was isolated from the cells and hydrolyzed to glucose in 1 N H$\text{SO}_4$ (12). The resulting solution was heated with Norit, filtered, and the clear effluent neutralized by passage through Duolite A 4 (OH$^-$). Glucose was analyzed by the Somogyi (13) and anthrone procedures (14). The purity of the glucose solutions was determined by paper chromatography in four different solvent systems: (a) 80% aqueous phenol, (b) n-butanol-ethanol-water (52:32:16), (c) collidine saturated with water, and (d) n-butanol saturated with water. Duplicate paper strips were sprayed for reducing sugars with aniline-phthalate according to Partridge (15) and for nonreducing sugars with silver nitrate and sodium hydroxide according to Trevyulan et al. (16). In each case only one spot corresponding to glucose was obtained.

An aliquot of the purified glucose was oxidized to carbon dioxide by the method of Van Slyke and Folch (17) and the remainder was degraded by fermentation with Leuconostoc mesenteroides according to the procedure of Bernstein and Wood (18). In this degradation C-1 of glucose is obtained as CO$_2$, C-2 and C-3 as ethanol, and C-4, C-5, and C-6 as lactic acid. Ethanol was ox-
Distribution of Cl$_4$ tails.

by chromatography on Celite and degraded to carbon dioxide.

Pyruvate-1-Cl$_4$ was isolated to acetic acid with dichromate. Lactic acid was distributed between the C-4 and C-6 positions.

Pyruvate-3-Cl$_4$ gave rise to lactate with about equal distribution of Cl$_4$ in the C-5 isomers and each of these was in the approximate ratio of 50:100:100. Pyruvate-1-, -2-, and -3-Cl$_4$ produced glucoses with a preponderance of activity in C-4, C-5, and C-6 atoms, respectively. Lactate-2-Cl$_4$ produced glucose with essentially all the activity being equally distributed between the C-4 and C-6 positions.

Two interesting features in the metabolism of the polymorphonuclear leukocytes have emerged from this study. The first is the direct incorporation of a 3-carbon unit into the glucose of glycogen and the second is the asymmetrical distribution of Cl$_4$ in the hexose unit.

The lack of significant Cl$_4$ randomization in the lactate isolated from the medium (Table II) as well as in the C-4, 5, and 6 unit. Acetate-1-Cl$_4$ labeled C-4 predominantly, whereas acetate-2-Cl$_4$ was preferentially incorporated into the C-4, C-5, and C-6 of the glucose in the approximate ratio of 50:100:100. Pyruvate-1-, -2-, and -3-Cl$_4$ produced glucoses with a preponderance of activity in C-4, C-5, and C-6 atoms, respectively. Lactate-2-Cl$_4$ predominantly labeled C-5 of the hexose molecule, whereas glyceraldehyde-1(3)-Cl$_4$ produced glucose with essentially all the activity being equally distributed between the C-4 and C-6 positions.

**DISCUSSION**

Two interesting features in the metabolism of the polymorphonuclear leukocytes have emerged from this study. The first is the direct incorporation of a 3-carbon unit into the glucose of glycogen and the second is the asymmetrical distribution of Cl$_4$ in the hexose unit.

The lack of significant Cl$_4$ randomization in the lactate isolated from the medium (Table II) as well as in the C-4, 5, and 6 units of the hexose (Table III) points to a direct route for the metabolism of pyruvate and lactate in polymorphonuclear leukocytes. Topper and Hastings (24), Lober et al. (25), and Landau et al. (26) in studies on rabbit and rat liver have shown that in the conversion of lactate-2-C$_4$ or pyruvate-2-C$_4$ to hexose nearly as
much C\(^{14}\) appears in position 6 as in 5. Their results are in accord with the operation of the dicarboxylic acid shuttle, whereby pyruvate is reductively carboxylated to malate (27), as shown in Fig. 1. After equilibration with the symmetrical fumarate, malate is oxidized to oxaloacetate which in turn gives rise to phosphoenolpyruvate by the Utter-Kurahashi reaction (28) as shown in Fig. 1. In the Embden-Meyerhof scheme the C-1, C-2, and C-3 of the hexose are from dihydroxyacetone phosphate, whereas the C-4, C-5, and C-6 arises from glyceraldehyde 3-phosphate. The probable explanation of these results is direct phosphorylation of pyruvate to phosphoenolpyruvate by way of a reversal of the pyruvic kinase reaction (30), although other explanations are also possible.

The distribution pattern of C\(^{14}\) in C-4, 5, and 6 which we observed is similar to that found by Lifson et al. (31) in the liver glycogen of fasted rats; in that with acetate-1-C\(^{14}\) the C\(^{14}\) is largely in C-4 and with acetate-2-C\(^{14}\) it is equal in C-5 and C-6 and is greater than in C-4 (Table III). These data and the labeling pattern obtained in the lactate (Table II) are consistent with the utilization of acetate in the polymorphonuclear leukocytes by way of the tricarboxylic acid cycle (Fig. 1).

C\(^{14}\)-glycerol, in contrast to labeled acetate, pyruvate, and lactate, contributes an appreciable percentage of its radioactivity to glycogen (Table I). Glycerol may enter the sequence of glycolytic reactions by phosphorylation to glycerol phosphate and then dehydrogenation to dihydroxyacetone phosphate. Triose phosphate isomerase placed C\(^{14}\) label into glyceraldehyde 3-phosphate. Since these steps do not involve pyruvic kinase or other major thermodynamic barriers, an appreciable conversion of glyceraldehyde 3-phosphate to glycogen is attained.

Perhaps the most unusual feature of the results in Table III is the high asymmetrical labeling of the six carbons of the glucose chain. In the Embden-Meyerhof scheme the C-1, C-2, and C-3 of the hexose are from dihydroxyacetone phosphate, whereas the C-4, C-5, and C-6 arises from glyceraldehyde 3-phosphate. Equilibration of the two trioses by triose phosphate isomerase and their combination by aldolase should yield a symmetrical hexose with equivalence between C-3 and C-4, C-2 and C-5, and C-1 and C-6 (24, 31, 32, 33). Asymmetrical distribution in the galactose of milk lactose has been demonstrated by Wood et al. (34) in studies with the perfused cow's udder. With acetate-1-C\(^{14}\) or propionate-1-C\(^{14}\), C-4 of the galactose moiety contained 3 times as much C\(^{14}\) as the C-3. In later studies Wood et al. (36) prepared glyceraldehyde 3-phosphate-1-C\(^{14}\) from phosphoglyceric acid-1-C\(^{14}\) and incubated it with fructose 6-phosphate and purified transaldolase. A rapid and exclusive labeling in C-4 of fructose 6-phosphate was obtained under these conditions. Srinivasan et al. (37) with the use of C\(^{14}\)-labeled glucose have also implicated the role of the above exchange mechanism in the biosynthesis of shikimic acid in Escherichia coli.

The high asymmetric labeling of glucose isolated from polymorphonuclear leukocytes (Table III) in these experiments may also be explained on the basis of the transaldolase exchange reaction. Thus by exchanging carbons 4, 5, and 6 of low activity fructose 6-phosphate with high activity glyceraldehyde 3-phosphate formed from C\(^{14}\)-labeled acetates, pyruvates, lactate, or glyceraldehyde 3-phosphate with a higher activity in C-4, 5, and 6 than in C-1, 2, and 3 is obtained as illustrated in Fig. 2.

Rosen (38) in tracer experiments with crystalline muscle aldolase has demonstrated an exchange between C-4, 5, and 6 of fructose 1,6-diphosphate and glyceraldehyde 3-phosphate. By exchanging low activity fructose 1,6-diphosphate with highly labeled glyceraldehyde 3-phosphate, a hexose diphosphate is produced with a higher content of label in C-4, 5, and 6 than in C-1,

![Fig. 1. Possible pathways for the incorporation of C\(^{14}\) into glycogen from labeled acetate, pyruvate, and lactate.](image_url)
2, and 3. This aldolase exchange mechanism could presumably produce the observed asymmetric pattern of label in leukocytes; however, the participation of fructose diphosphatase is required for the conversion of C14 fructose 1,6-diphosphate to fructose 6-phosphate and hence to glycogen. In view of the very low fructose diphosphatase activity in the rabbit polymorphonuclear leukocyte, it is proposed that the transaldolase rather than the aldolase reaction may be the predominant exchange mechanism responsible for the observed asymmetric distribution of label in the polymorphonuclear leukocyte.

No attempt has been made to demonstrate net synthesis of glycogen from the two and three carbon substrates used in these experiments. The C14 activity found in glycogen may be a consequence of at least two exchange mechanisms, viz. pyruvic kinase and transaldolase, operating in leukocyte metabolism.

SUMMARY

Polymorphonuclear leukocytes, obtained from rabbits by peritoneal irritation were incubated with C14 labeled substrates in vitro. The lactate of the medium and the glycogen of the cells were isolated, purified and the distribution of label in each compound was determined by degradation.

Acetate, pyruvate, and lactate are incorporated to a very small extent into leukocyte glycogen, but glycerol is incorporated to an appreciable extent.

The degradation data suggest that the Krebs cycle is involved in the incorporation of acetate carbon into glycogen. The C14 of pyruvate and lactate is not randomized extensively during the conversion to glycogen and could follow a direct path. This may involve a reversal of the pyruvic kinase reaction. The high incorporation of glycerol into glycogen is due to its introduction at the triose phosphate level.

With all substrates studied, most of the C14 activity (86 to 94%) was incorporated into C-4, 5, and 6 of the hexose unit of glycogen. The highly asymmetrical distribution of label is explained by the transaldolase exchange mechanism.

The C14 activity incorporated into glycogen from labeled acetate, pyruvate, lactate, and glycerol may not necessarily indicate net synthesis, but rather may be a consequence of exchange mechanisms, viz. pyruvic kinase and transaldolase, operating in leukocyte metabolism.

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