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 $V_{max}$ of a number of these enzymes. The results indicate that 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 defibrination, 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 1 N HCl.

**Radioactive Materials**—Sodium acetate-1- and -2-$^{14}C$ and zinc lactate-2-$^{14}C$ were obtained from Volk Radiochemical Company. Sodium pyruvate-1-, -2-, and -3-$^{14}C$ were purchased from Nuclear-Chicago Corporation. Glycerol-1(3)-$^{14}C$ (m-glycerol-1-$^{14}C$) 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 X $10^9$ 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$_2$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$_2$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-phthaleate according to Partridge (15) and for nonreducing sugars with silver nitrate and sodium hydroxide according to Trevelyan 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-
Carbon dioxide obtained from the degradations of lactic acid was converted to barium carbonate and its isotope content measured with a windowless gas flow counter. Equivalence between the two counters was obtained by employing a conversion factor.

RESULTS

The percentage incorporation of C\textsuperscript{14} into glycogen and lactic acid from the substrates under study is shown in Table I. Only one to five thousandth of a % of the initial radioactivity in labeled acetates, pyruvates, and lactate entered leucocyte glycogen. Glycine, serine, formate, and formaldehyde were even less adequate as precursors of glycogen. Incubation of as much as 100 \mu C of these compounds with rabbit polymorphonuclear leukocytes has yielded no detectable radioactivity in the isolated polysaccharide. In contrast to the above mentioned substrates, C\textsuperscript{14} glyceraldehyde produced an appreciable labeling of the white cell glycogen. Lactate from these experiments had, with the exception of the glyceraldehyde experiment, a considerably higher concentration of C\textsuperscript{14} than the corresponding glycogen samples. With glyceraldehyde the glycogen of the cell and the lactate in the medium had about the same total counts.

The distribution pattern of C\textsuperscript{14} in the lactate carbon atoms is presented in Table II. With acetate-1-C\textsuperscript{14} as the labeled substrate all the C\textsuperscript{14} was recovered in the carboxyl group of the lactate. Acetate-2-C\textsuperscript{14} gave rise to lactate with about equal distribution of C\textsuperscript{14} in the \( \alpha \) and \( \beta \) carbons and each of these were in turn more highly labeled than the carboxyl carbon atom. Lactate isolated from experiments with C\textsuperscript{14}-labeled pyruvates, lactate, and glycerol had essentially all the activity in the position corresponding to that of the original C\textsuperscript{14} tagged substrate.

Although a very small percentage of the original acetate, pyruvate, and lactate radioactivity appeared in leucocyte glycogen, there was sufficient C\textsuperscript{14} for accurate determinations. The reliability of the degradation procedure was demonstrated by good agreement between the sum of the activities of the individual carbon atoms as obtained by degradation and that of the original hexose. The degradation data are shown in Table III. With all of the labeled substrates used, 86% to 94% of the hexose C\textsuperscript{14} activity was located in the C-4, 5, and 6 unit. Acetate-1-C\textsuperscript{14} labeled C-4 predominantly, whereas acetate-2-C\textsuperscript{14} 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-C\textsuperscript{14} produced glucoses with a preponderance of activity in C-4, C-5, and C-6 atoms, respectively. Lactate-2-C\textsuperscript{14} predominantly labeled C-5 of the hexose molecule, whereas glyceraldehyde-1(3)-C\textsuperscript{14} 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 C\textsuperscript{14} in the hexose unit.

The lack of significant C\textsuperscript{14} 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. Topp and Hastings (24), Lorber 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\textsuperscript{14} or pyruvate-2-C\textsuperscript{14} to hexose nearly as
much C¹⁴ 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 phosphoenol pyruvate by the Utter-Kurahashi reaction (28). In our experiments, the lack of randomization of C¹⁴ in C-5 and C-6 resembles the distribution pattern observed by Hiatt et al. (29), in the glycogen of rat diaphragm. The probable explanation of these results is direct phosphorylation of pyruvate to phosphoenol pyruvate by way of a reversal of the pyruvic kinase reaction (30), although other explanations are also possible.

The distribution pattern of C¹⁴ 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-C¹⁴ the C¹⁴ is largely in C-4 and with acetate-2-C¹⁴ 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).

C¹⁴-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 dihydroxy acetone phosphate. Triose phosphate isomerase placed C¹⁴ label into glyceraldehyde 3-phosphate. Since these steps do not involve pyruvic kinase or other major thermodynamic barriers, an appreciable conversion of glycerol 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 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-C¹⁴ or propionate-1-C¹⁴, C-4 of the galactose moiety contained 3 times as much C¹⁴ as the C-2. In later studies Wood et al. (36) with the use of the intact cow, found that glycerol-1-(3)-C¹⁴ likewise predominated labeled C-4 and C-6 of the galactose. The transaldolase exchange reaction was proposed to account for the observed labeling pattern. In a further confirmation of the role of the transaldolase reaction in these exchange mechanisms, Wood et al. (36) prepared glyceraldehyde 3-phosphate-1-C¹⁴ from phosphoglyceric acid-1-C¹⁴ 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 C¹⁴ 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 C¹⁴-labeled acetates, pyruvates, lactate, or glycerol a hexose with a higher activity in C-4, 5, and 6 than in C-1, 2, and 3 is obtained as illustrated in Fig. 2.

Fig. 2. Transaldolase exchange mechanism. Bold face carbon (C) indicates C¹⁴ labeled atom.
No attempt has been made to demonstrate net synthesis of glycogen from the two and three carbon substrates used in these experiments. The C4 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 C4 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 C4 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 C4 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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**REFERENCES**


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\(^1\) E. P. Noble and R. L. Stjernholm, unpublished observations.
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