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EFFECT OF SUBLETHAL DOSES OF CYANIDE ON GLUCOSE CATABOLISM*

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Abstract—The effect of cyanide on the metabolic degradation of glucose in the mouse was investigated by comparing the rate and extent of production of respiratory $^{14}CO_2$ from specifically labeled carbon atoms of glucose, gluconate and glucuronate. Glucose in the untreated animals was metabolized via three pathways: Embden–Meyerhof–Parnas pathway and tricarboxylic acid cycle (EMP–TCA), pentose phosphate pathway and glucuronate pathway. Administration of 5 mg/kg of potassium cyanide, a sub-lethal dose, increased catabolism of carbohydrates by the pentose phosphate pathway with a decline in utilization of the EMP–TCA cycle and glucuronate pathway. These results suggest that chronic exposure to cyanide may induce marked alterations in normal carbohydrate metabolism, and the possible relationship to specific pathological conditions is discussed.

A number of metabolic processes are known to be influenced by cyanide. Cyanide inhibits cytochrome oxidase which results in an alteration of a complex series of oxido-reductive reactions in the cell. Other systems inhibited by cyanide include amylase synthesis [1] and enzymes requiring pyridoxal phosphate as a coenzyme [2]. In addition, cyanide has been reported to stimulate glycerol-l-phosphate dehydrogenase [3, 4].

Inactivation of cytochrome oxidase by cyanide results in a shift of aerobic to anaerobic metabolism [5–9] accompanied by a marked accumulation of lactate. The concentration of ATP and phosphocreatine decreases and ADP increases. This modification of normal metabolism may result in an increased utilization of alternate pathways and/or induction of side pathways in an attempt by the cell to maintain a balanced redox state and energy pool. In the present study, the nature of the pathways for glucose catabolism was investigated in the normal and cyanide-treated animal by radiorespirometric experiments. Specifically labeled carbon-14 glucose, gluconate and glucuronate were administered to mice, and respiratory excretion of $^{14}CO_2$ produced from the metabolic conversion of labeled substrate was monitored. Analysis of the excretion patterns of labeled carbon dioxide permitted a qualitative determination of pathway utilization in the normal and cyanide-treated mice.

MATERIALS AND METHODS

Specifically labeled carbon-14 glucose and glucuronic-6-^{14}C acid, sodium salt, were obtained from the New England Nuclear Corp. (Boston, Mass.) and gluconic-1-^{14}C acid, sodium salt, from the International Chemical Nuclear Corp. (Irvine, Calif.). Unlabeled glucose was analytical grade produced by Mallinckrodt Chemical Works (St. Louis, Mo.) and glucuronic acid, sodium salt (grade II), and gluconic acid, potassium salt (grade III), were obtained from Sigma Chemical Co. (St. Louis, Mo.). 2,5-Diphenyloxazole (PPO) and 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl POPOP) (scintillation grade) were purchased from the Packard Instrument Co. (Downers Grove, Ill.). Methanol and ethylene glycol were analytical grade reagents from the J. T. Baker Chemical Co. (Phillipsburgh, Pa.). 1,4-Dioxane (J. T. Baker Chemical Co.) was liquid scintillation counting grade.

Isotopically labeled glucose was mixed with nonradioactive anhydrous glucose so that the specific activity was approximately 0·01 μCi/mg of glucose (Table 1). Specific activity of glucose-3(4)-^{14}C was approximately 0·006 μCi/mg. Gluconate-1-^{14}C and glucuronate-6-^{14}C contained about 0·001 μCi nonradioactive substrate. These mixtures were diluted with distilled water so that the solutions contained glucose in a concentration of 200 mg/1·0 ml or gluconate and glucuronate in concentrations of 20 mg/1·0 ml. All isotopic solutions were stored frozen in rubber-stoppered serum vials. Solutions were injected intraperitoneally in a volume of 0·5 ml in all animals. Radioactivity was determined in a Packard Tri Carb model 3320 liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) employing Bray's solution [10] (naphthalene, 60 g; PPO, 4 g; dimethyl POPOP, 0·2 g; methanol, 100 ml; ethylene glycol, 20 ml; 1,4-dioxane, 375 ml). Potassium cyanide was administered intraperitoneally at a dose of 5 mg/kg in a volume of no more than 1 per cent of body weight. Potassium cyanide solutions were prepared fresh each day.

Male Swiss–Webster mice (Horton Animal Laboratories, Oakland, Calif.) were housed in air conditioned quarters maintained at 21–22° and were allowed free access to standard commercial diet and water *ad lib.* Animals weighing 30 ± 2 g were fasted 24 hr before initiation of the experiment. The labeled compound and potassium cyanide were administered concurrently,

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Table 1. Labeled substrates employed in radiorespirometric experiments

Substrate	No. of mice tested	Amount injected (μCi)	Substrate injected (mg)
Glucose-1-^{14}C	8	1·24	100
Glucose-2-^{14}C	8	0·95	100
Glucose-3(4)-^{14}C	10	0·58	100
Glucose-6-^{14}C	8	0·98	100
Gluconate-1-^{14}C	9	0·84	10
Glucuronate-6-^{14}C	8	1·12	10

and the animal was placed immediately in a 425-ml Roth metabolism chamber with minor modifications as described by Wang [11].

Respiratory excretion of $^{14}CO_2$ was determined continuously over a 6-hr period with a Cary 32 vibrating reed electrometer with a 250-ml ion chamber (Applied Physics Corp., Monrovia, Calif.) employing an air flow rate of 250 ml/min [11]. Calibration was accomplished by sweeping a known amount of $^{14}CO_2$ through the ion chamber, and the ion current produced was recorded on a rectilinear recording millammeter (Texas Instrument Inc., Houston, Tex.). A calibration curve in terms of μCi/unit area was established by measuring the area under the curve in triplicate by means of a compensating polar planimeter.

RESULTS

Respiratory excretion of labeled carbon atoms of glucose as $^{14}CO_2$ at 30-min intervals is shown in Fig. 1. Each curve represents the mean of four or more mice, and variation among the replicated experiments was approximately 6 per cent. Peak excretion of $^{14}CO_2$ for each glucose label was observed during the 60-min monitoring interval. At the end of the 6-hr experimental period, the 30-min yields decline to less than 2 per cent of administered activity. Analysis of interval yields of the $^{14}CO_2$ excretion showed that the production of $^{14}CO_2$ from C-3(4) was more rapid than any other glucose carbon atoms. During the 60-min period, production of $^{14}CO_2$ from C-6 exceeded that from C-2, and the production from C-1 was the lowest. Cumulative yields of labeled carbon dioxide (Fig. 2 and Table 2) indicate C-3(4) was excreted to the greatest extent followed by C-6 and C-2. Recovery rates of C-1 were lower than the other labels.

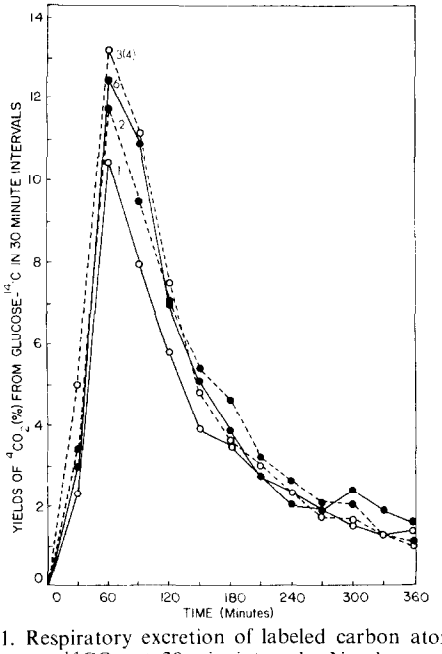

Fig. 1. Respiratory excretion of labeled carbon atoms of glucose as $^{14}CO_2$ at 30-min intervals. Numbers refer to specifically labeled carbon atoms on glucose.

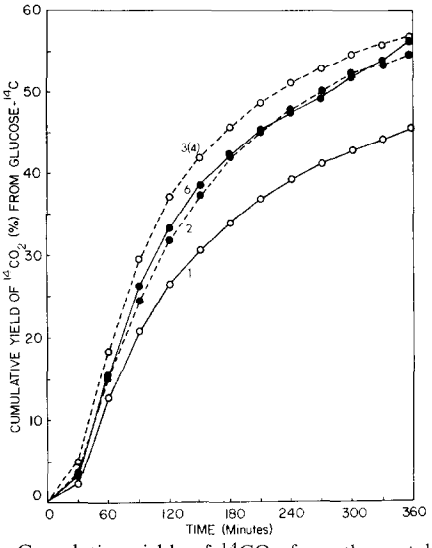

Fig. 2. Cumulative yields of $^{14}CO_2$ from the metabolism of specifically labeled glucose. Numbers refer to specifically labeled carbon atoms on glucose.

Administration of potassium cyanide (5 mg/kg) altered the normal excretion patterns of $^{14}CO_2$ (Fig. 3). Extensive recovery of C-1 of glucose in respiratory CO_2 is shown in the cumulative yields (Fig. 4 and Table 2). The C-3(4) recovery was decreased more extensively than that of the other labeled carbon atoms,

Table 2. Cumulative 360-min yields of $^{14}CO_2$ from specifically labeled carbon atoms*

Substrate	Per cent recovery Controls	KCN treatment
Glucose-1-C	45	42
Glucose-2-C	54	31
Glucose-3(4)-C	57	26
Glucose-6-C	56	39
Gluconate-1-C	16	31
Glucuronate-6	12	7

* Yields are expressed as per cent recovery of administered carbon-14.

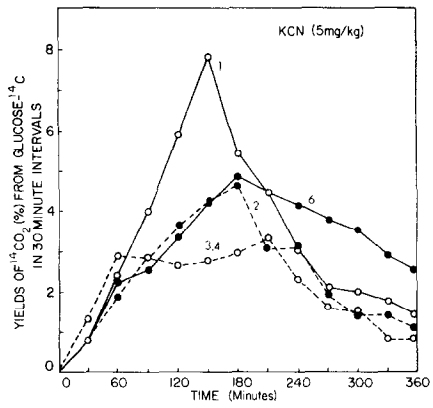

Fig. 3. Respiratory excretion of labeled carbon atoms of glucose as $^{14}CO_2$ at 30-min intervals after the administration of potassium cyanide (5 mg/kg). Numbers refer to specifically labeled carbon atoms on glucose.

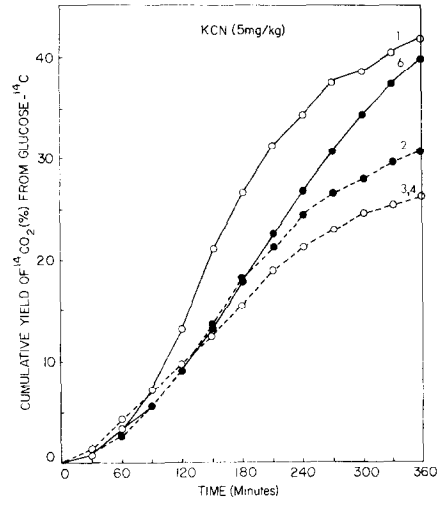

Fig. 4. Cumulative yields of $^{14}CO_2$ from the metabolism of specifically labeled glucose after the administration of potassium cyanide (5 mg/kg). Numbers refer to specifically labeled carbon atoms on glucose.

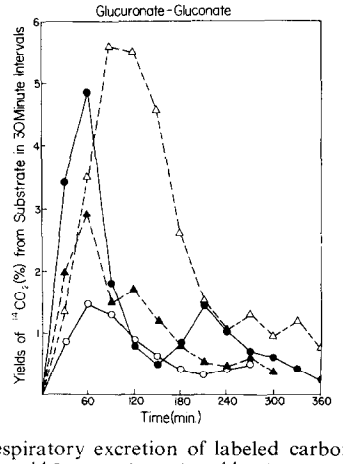

Fig. 5. Respiratory excretion of labeled carbon atoms of glucuronic-6-^{14}C and gluconic-1-^{14}C in 30-min intervals in control animals and after the administration of potassium cyanide (5 mg/kg). Key: ●——●, glucuronic-6-^{14}C; ○——○, glucuronic-6-^{14}C + KCN; ▲——▲, gluconic-1-^{14}C; △——△, gluconic-1-^{14}C + KCN.

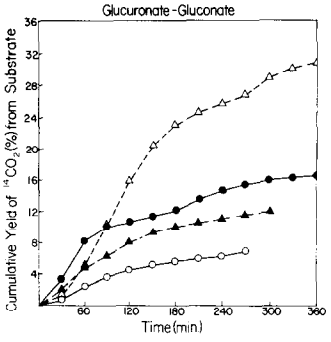

Fig. 6. Cumulative yields of $^{14}CO_2$ from the metabolism of glucuronic-6-^{14}C and gluconic-1-^{14}C in controls and after the administration of potassium cyanide (5 mg/kg). Key: ●——●, glucuronic-6-^{14}C; ○——○, glucuronic-6-^{14}C + KCN; ▲——▲, gluconic-1-^{14}C; △——△, gluconic-1-^{14}C + KCN.

strongly suggesting an alteration in metabolic pathway(s).

Respiratory excretion of C-6 of glucuronate and C-1 of gluconate in normal and KCN-treated animals is shown in Fig. 5. In untreated mice, C-6 of glucuronate was excreted more extensively than C-1 of gluconate. Peak excretion interval for each labeled carbon was during the 60-min period. Also, cumulative excretion was greater for the glucuronate label (Fig. 6). After cyanide treatment, C-1 gluconate recovery was greatly increased above the excretion rate of the normal animal, as reflected in both interval and cumulative yield curves (Figs. 5 and 6). On the other hand, C-6 recovery of the glucuronate was decreased in the cyanide-treated mice as compared to the untreated animal, suggesting a quantitative and/or qualitative change in catabolic pathway utilization (Table 2).

DISCUSSION

Differences in rates and extents of production of $^{14}CO_2$ from individual carbon atoms of glucose reflect the sequential biochemical reactions traversed by the specifically labeled substrate. Analysis of the respiratory excretion pattern of $^{14}CO_2$ can be utilized to characterize the sequence of biochemical pathways traversed by a substrate in a biological system if carbon dioxide is an end product of the reaction(s) [11]. In the present experiment, differences in rate and extent of excretion patterns of $^{14}CO_2$ are not due to absorption, since the entire glucose molecule is involved in this process.

Exclusive metabolism of glucose by the Embden–Meyerhof–Parnas pathway (EMP) and tricarboxylic acid cycle (TCA) would be reflected by a prompt and extensive recovery of C-3(4) atoms of glucose in respiratory CO_2 [11, 12]. Preferential conversion (greater or earlier conversion) of C-2 over C-1 and C-6 would be observed, and production of C-1 and C-6 should be equal if metabolism is exclusively via way of the EMP–TCA pathway. In the present investigation, the role of the EMP pathway in the normal animal is reflected by a greater extent and rate of production of C-3(4). Yields from C-3(4) can be used as a direct measure of the EMP participation in glucose catabolism. The results suggest that approximately 57 per cent of the

injected glucose was metabolized via the EMP pathway. However, the rate of appearance of C-6 was greater than C-2, suggesting the presence of other pathways or mechanisms in addition to the EMP–TCA pathway.

Preferential conversion of C-6 of glucose to CO_2 over that of C-2 suggests operation of the glucuronic acid cycle pathway [13]. The operation of the glucuronic acid pathway is further confirmed by radiorespirometric data obtained from mice injected with glucuronate-6-^{14}C. Carbon-6 of glucuronate was readily converted to carbon dioxide. Evidence for operation of the pentose phosphate pathway is given by data obtained from mice injected with gluconate-1-^{14}C. The average yield of $^{14}CO_2$ from C-1 of gluconate was 16 per cent of injected activity, indicating gluconate is metabolized by mice. According to Gunsalus *et al.* [14], the conversion of glucose to phosphogluconate is an irreversible reaction. Therefore, it does not seem likely that C-1 of gluconate could have been converted to carbon dioxide directly by way of the EMP–TCA route.

The control mice appear to utilize three pathways for glucose utilization. The EMP–TCA pathway appears to be major, accounting for about 57 per cent of the total amount of glucose metabolized. The glucuronic acid and pentose phosphate pathways are minor. Estimates of the percentage of glucose metabolized by the minor pathways cannot be made from the data.

The marked decrease of the respiratory excretion of C-3(4) in potassium cyanide-treated animals indicates a reduction of catabolism via way of the EMP–TCA pathway. The amount of glucose traversing the EMP pathway was reduced by 54 per cent. Since the formation of carbon dioxide from C-2 is presumably due exclusively to TCA decarboxylation processes, the 42·5 per cent decrease in C-2 yield in cyanide-treated animals suggests also a reduction in TCA involvement.

It was noted that the yield from C-1 of glucose did not change significantly in the cyanide-treated animals as compared to controls. If conversion of C-1 to carbon dioxide had occurred only by way of the TCA cycle, there should have been a decrease in the yield. The magnitude of the decrease should have been about the same as that for C-2. Thus, C-1 yields from TCA activity must have been reduced, and the yields from the pentose phosphate pathway were increased. This is confirmed by the preferential conversion of C-1 glucose to CO_2 over that of C-2 in mice receiving potassium cyanide.

Stimulation of the pentose phosphate pathway was confirmed in mice receiving potassium cyanide and administered gluconate-1-^{14}C. The gluconate data suggest that there was about a 100 per cent increase in pentose phosphate pathway utilization.

Glucuronate-6-^{14}C conversion of $^{14}CO_2$ was decreased, indicating a decline in operation of the glucuronic acid pathway in cyanide-treated mice. This was confirmed by a decrease in C-6 yields.

The present investigation suggests a sublethal dose of cyanide increased catabolism of carbohydrates by way of the pentose phosphate pathway, accompanied by a decline in EMP–TCA and glucuronic acid pathways. Since operation of the pentose phosphate pathway generates reducing power (NADPH), this change in pathway catabolism may reflect a compensating mechanism of the cell to maintain a balanced redox state which is an important factor in determining

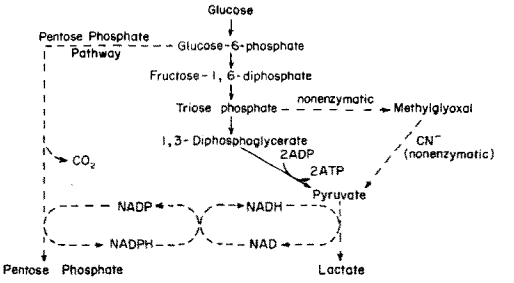

Fig. 7. Proposed scheme of carbohydrate metabolism in the presence of cyanide. Broken lines represent metabolic pathways activated and/or increased in utilization by cyanide.

which metabolic reactions may take place at any one time. Baxter and Hensley [15] demonstrated that administration of cyanide to rats increased hepatocyte cytoplasmic NAD/$NADH_2$ ratio. Numerous investigators have reported that cyanide induces a shift of aerobic to anaerobic metabolism resulting in a marked accumulation of lactate [5–9]. These observations suggest an increased conversion of pyruvate to lactate at the expense of NADH generated at the triose oxidation step of glycolysis. Experiments *in vitro* suggested that cyanide catalyzes a nonenzymatic conversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate to pyruvate [16]. This nonenzymatic production of pyruvate may involve methylglyoxal (pyruvate aldehyde: CH_3COCHO) as an intermediate. The metabolism of triose phosphates to lactate via methylglyoxal would produce 1 mole NADH/mole of triose phosphate, resulting in an imbalance of reduced nucleotides [15]. In order for the cell to compensate this altered redox state, perhaps the pentose phosphate pathway is stimulated, providing a source of NADPH which can reduce NAD by means of a transhydrogenase enzyme (Fig. 7).

A number of pathological conditions related sublethal doses of cyanide may be associated with such alterations in intermediary metabolism. Among the pathological conditions produced by low concentrations of cyanide are: lathyrism [17], neuropathy, demyelination and cyanide encephalopathy [18, 19]; tobacco amblyopia and certain other effects of cigarette smoking have also been attributed to cyanide [20, 21]. Additional metabolic investigations in long-term cyanide administration, such as cigarette smoking or environmental pollutants, are warranted. Chronic studies may disclose even a greater modification of intermediary metabolism induced by sublethal exposure to cyanide.

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