Metabolism of $NaHC^{14}O_3$: Effects of Anesthesia and Route of Dosage.* (22191)

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A number of workers(1,2) have studied health physics aspects of $NaHC^{14}O_3$ metabolism and others(3,4) have shown bicarbonate carbon to be fixed in a variety of metabolites. We were unable to find adequate information in the literature on early times of the time course of $C^{14}O_2$ excretion or of effects of anesthesia upon this excretion. The effect of a further variable, route of dosage, also does not appear to have been reported. For adequate interpretation of the fate of labeled metabolites that are oxidized, it is necessary to know the fate of $C^{14}O_2$ so produced. In a following study important use is made of the time course of $C^{14}O_2$ excretion following acetate dosage.

Methods. Rats were given tracer $NaHC^{14}O_3$ under a variety of conditions and respired $C^{14}O_2$ and $C^{12}O_2$ were measured. Following multiple dosage of the $HC^{14}O_3^-$ the rats were sacrificed and tissue lipides analyzed for C^{14} activity. Sprague-Dawley, adult, male, 200-g white rats trained to feeding as before(5) were used exactly one hour postprandially. Following injection, the rats were placed in the metabolism chamber of the apparatus(6) and respiratory $C^{14}O_2$ activity data collected from the decimal scaler component. Occasional one-minute CO_2 samples were collected. The total CO_2 and $C^{14}O_2$ exhaled was collected and the amount and activity of the CO_2 was determined. Animals anesthetized were given 3.5 mg/100 g body weight of Nembutal. The anesthesia was given 45 minutes after start of the one-hour eating period, which was thus 15 minutes before the tracer bicarbonate was given. Radiobicarbonate was prepared by acidification of Oak Ridge $BaCO_3$ and absorption of the released CO_2 in NaOH. The pH of the solution was 8.2 and contained 0.38 mg $NaHCO_3$/ml with an activity of 10.84 μc/ml; 0.4 ml was the usual dose. Aliquots of the standard $NaHCO_3$ solution and respiratory CO_2 samples collected in alkali were directly plated(7) and counted under a thin end window tube as before(8). Because of low activities found in tissue samples, the fatty acids were directly plated, the cholesterol was plated as the digitonide and liver glycogen was also directly plated. Counting rates were corrected to "infinite thickness" by the application of appropriate factors previously determined in this laboratory. Specific activity figures listed below are on the order of one-tenth of the specific activities obtainable at "infinite thinness." A total of 9 experiments was conducted on 2 rats. Since each animal was subjected to the 3 conditions studied, "internal control" of experimental conditions was maintained. Intravenous injections were made in the saphenous vein at the junction of saphenous and lateral plantar veins. This route of injection permits a rapid yet quantitative administration of label. Following intravenous injection of HCO_3^- the animal was put into the metabolism system with dispatch because of the rapid rate of $C^{14}O_2$ excretion. The greater part of the bicarbonate dose having been exhaled in 3 hours allowed us to reuse the rats 24 to 48 hours after previous dosage. Forty-eight hours following the second dose of bicar-

TABLE I. Dosage Schedule for the Two Rats Receiving Tracer Bicarbonate.

Exp.	Animal No.	Dose, ml	Route	Anesthesia
1	V5-1	.4	I.P.	—
2	2	"	"	+
3	3	"	"	—
4	4	"	I.V.	+
5	V6-1	.8	I.P.	—
6	2	.4	"	+
7	3	"	"	—
8	4	"	"	—
9	5	"	I.V.	+

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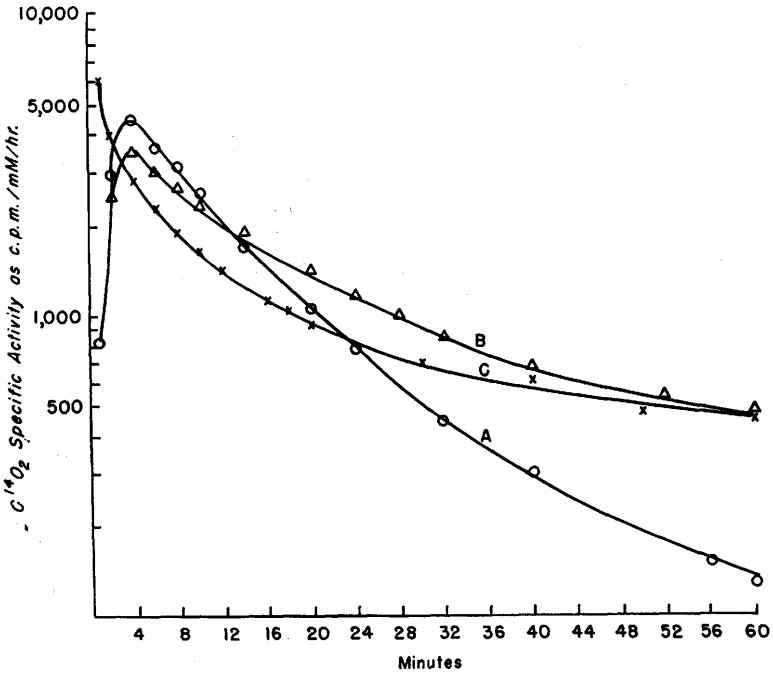

FIG. 1. Respiratory $C^{14}O_2$ activities following $NaHC^{14}O_3$ dosage. Curve A, avg of 4 experiments with 2 rats, 0.4 ml $NaHCO_3$ intraperitoneally. Curve B, as with the experiments of A but with rats anesthetized with Nembutal. Curve C, avg of 2 experiments in which anesthetized animals were given the tracer $NaHC^{14}O_3$ intravenously.

bonate the animals were placed in the metabolism chamber and respiratory $C^{14}O_2$ activity monitored. No activity above background was detected.

Results. Table I presents the details of the nine experiments from which time-course CO_2 data were obtained. Exp. 1 and 3 represent duplicate intraperitoneal, nonanesthetized runs on the first animal and Exp. 7 and 8 similar duplicate runs on second animal, giving a total of 4 trials of these conditions. Exp. 2 and 4 tested the effects of Nembutal upon $C^{14}O_2$ excretion after intraperitoneal dosage. Runs 4 and 9 tested the combined effects of Nembutal and intravenous dosage. The effect of dosage size was tested in Exp. 1 and 5.

The c.p.m. obtained from the decimal scaler(6), were converted to specific activities by dividing the actual count by the mM CO_2 produced per hour. This latter figure was obtained from the amount of CO_2 collected during the entire experiment. In each experiment 2, one-minute samples of CO_2 were collected and analyzed separately. The amount of CO_2 produced per minute measured by these samples agreed, within the usual range of such values, with the average CO_2 figures obtained from the total CO_2 collection.

The specific activity values used as the ordinate of Fig. 1 represent c.p.m. per mM CO_2 produced per hour, the counts used, being those recorded by the gas flow counting of the system described. These counting rates can be converted to equivalent infinitely thick $BaCO_3$ counting rates by multiplying by a factor of 5.3.

In Fig. 1 are presented the 3 curves that represent average results obtained from experiments detailed in Table I. Curve A represents the time-course of respired $C^{14}O_2$ following intraperitoneal dosage without anesthesia and curve B the effect of Nembutal anesthesia upon the $C^{14}O_2$ excretion. Following the intraperitoneal dosage both with and without anesthesia, $C^{14}O_2$ specific activities quickly increased and reached a maximum in about 6 minutes, demonstrating the rapidity

of peritoneal absorption processes. Attainment of similar maxima at similar times suggests that the intraperitoneally administered Nembutal did not greatly affect HCO_3^- absorption from the peritoneal space. The $C^{14}O_2$ specific activities decreased following the maxima but plots of log s.a., $\log\frac{1}{\text{s.a.}}$, or $\log\frac{1}{\text{s.a.}^2}$ fail to reveal a straight line function for this decay, indicating the complex nature of the simultaneous distribution and excretion of the labeled bicarbonate. In the period immediately following the maxima, 7 minutes were required to reduce by ½ the s.a. of the intraperitoneal animals not anesthetized while 11 minutes were required for the anesthetized animals to reduce their $C^{14}O_2$ specific activities to one-half. Also, the anesthetized animals excreted only some 85% as much of the dose as did the nonanesthetized animals in the first hour. The decreased "turnover" of the HCO_3^- in the anesthetized rats is consistent with the slowing of respiration associated with Nembutal anesthesia. Upon awakening, in the 40- to 60-minute period, the rat treated with Nembutal struggles briefly and hyperventilates, which results in a sharp upward deflection in CO_2 specific activities. Following this, the respiratory rate of the rat slows and approximates that of the non-Nembutalized animal, for the slopes of the CO_2 specific activity curves become similar.

Curve C of Fig. 1 presents the average of the $C^{14}O_2$ time-course curves of the 2 experiments in which anesthetized animals were given bicarbonate intravenously. $C^{14}O_2$ appears in the respired air with such rapidity that it is difficult to make an accurate reading of the scaler at one minute. At this early time the respiratory CO_2 specific activity was found to be maximal, as might be expected from the probable maximum s.a. of blood HCO_3^-. The shape of the time-course curve plotted as C is similar in shape to curve B, confirming the effect of Nembutal anesthesia upon CO_2 turnover as seen in the intraperitoneally injected preparations.

Following the collection of CO_2 data in 4 experiments on animal V5 and in 5 experiments on animal V6, the rats were fractionated as previously described(5) and total saponifiable and nonsaponifiable lipide fractions isolated from liver, gut, carcass and skin. Liver glycogen was isolated from V5 and directly plated and counted as were the lipide fractions. The C^{14} activity of the lipides was extremely low and the data is thus not presented in detail. In the cholesterol (nonsaponifiable) fractions, the maximum per cent incorporation found was only 0.004. The maximum incorporation found for fatty acids (saponifiable fraction) was 0.05%. Compared to equivalent dosage with acetate-1-C^{14} in one hour postabsorptive animals, the cholesterol and fatty acid incorporation was less than 0.005 of that usually found. It is clear that the $C^{14}O_2$ released from tracer dosage, even from rapidly metabolized labeled substrates such as acetate, does not to a significant degree(4) label body fatty acids or cholesterol.

The liver glycogen of V5 after the 4 doses of tracer bicarbonate contained about 0.01% of the label. This figure has little absolute meaning because of the multiple nature of the dosage and the period of time elapsed during the 4 experiments.

Summary. The time-course of respiratory $C^{14}O_2$ has been followed after intraperitoneal and intravenous dosage of $NaHC^{14}O_3$ into anesthetized and non-anesthetized rats. Nembutal has been shown to affect the turnover of injected bicarbonate through its depression of respiration. The anesthetic did not slow peritoneal absorption. The multiple bicarbonate dosage used did not lead to appreciable labeling in tissue lipids, and the contribution of $C^{14}O_2$ to secondary labeling reactions can under these conditions be ignored. Liver glycogen "fixed" a small but measurable amount of the C^{14} label.

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Hyperglycemia and Increased Liver Glycogen in Rats After X-Irradiation. *† (22192)

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It is well established that whole-body x-irradiation of rats results in an increase in level of liver glycogen(1-4). On the other hand it has been reported that liver glycogen of rabbits is markedly reduced following x-irradiation(5). Although x-irradiation raises liver glycogen level of rats and decreases that of rabbits, blood sugar levels in both animals are increased by exposure to x-irradiation. Kohn(6) found that in rats irradiated with 600-900 r, plasma glucose level rises within 2 to 3 days and McKee(4) has found, on the first day after irradiation, an increase in blood sugar levels of rats exposed to 1500 r. Steadman and Grimaldi(7) observed a hyperglycemia in rabbits following x-irradiation. This hyperglycemia increased with increasing dose and at every dose (500 to 2000 r) reached a maximum 4 hours after irradiation, thereafter declining to normal levels within 24 hours. Hyperglycemia was completely prevented by insulin. Therefore, these authors postulated that irradiation causes a mobilization of liver glycogen into glucose and that the normal supply of insulin in the rabbit is not sufficient to prevent hyperglycemia. Since irradiation increases liver glycogen levels in the rat, the question arises as to the reason for hyperglycemia. Before an attempt was made to answer this question, it appeared that a detailed study of the relationship of liver glycogen and blood sugar levels to dose and time after irradiation would be of value. Data on these points are presented in this paper.

Methods. Animals. Sprague-Dawley female rats about 10 weeks of age were used. In each experiment, animals of uniform weight were divided into groups of either 4 or 5 rats each. *Radiation technics.* Radiation source was Westinghouse X-ray machine of 250 KVP and 15 ma, with filter of 0.5 mm copper and 1 mm aluminum. All doses were delivered at 24.5 per minute at a target-to-skin distance of 40 inches. During irradiation rats were restrained in acetate-plastic boxes and slowly rotated under the beam to insure uniform exposure(8). Non-irradiated, control animals were caged in the same manner for the same period of time. *Experimental design.* In Exp. 1 only liver glycogen was determined. In Exp. 2 both liver glycogen and blood sugar levels were determined, whereas in Exp. 3 only blood sugar levels were measured. Rats subjected to whole-body x-irradiation were fasted for 24 hours, exposed to single x-ray dose and the fast continued. Doses administered were 500,

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