Determination of Lactate Kinetics in the Human Analysis of Data From Single Injection vs. Continuous Infusion Methods (36284)

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Serious question has been raised about the applicability of the single tracer injection method for the determination of metabolite (lactate) kinetics in the intact animal (dog) (1). Forbath *et al.* note that calculation errors may easily arise in the analysis of data comprising the terminal exponential component of the specific activity time curve, when the substrate in question undergoes very rapid turnover in the plasma. In preliminary attempts to obtain precise early data in a primed infusion study of lactate kinetics in the human, we noted that the final exponential component of the single injection lactate study was a very poor guide to the kinetics of the system. In further investigation into the relationship of the single tracer injection *vs.* primed tracer infusion of lactate in the human we have resorted to a curve-integration analysis as proposed by Steele (2) and as outlined by Tait and Burnstein (3). In the present report we present the results of data analysis from studies of lactate kinetics by both the single tracer injection and primed-tracer infusion techniques carried out in four normal individuals. These results demonstrate that comparable estimates of lactate turnover rates may be obtained by either technique.

Methods. Selection of subjects. Volunteer subjects were selected from a nondiabetic population awaiting elective surgery.

Experimental design and procedures. Experiments were begun at approximately 9:00 AM after an overnight fast of 12 hr. At zero time of each study an injection or infusion of 25 μCi of uniformly labeled ^{14}C-L-(+)-lactic acid[1] was begun according to the appropriate protocol. Following the administration of tracer substrate, blood samples were collected as depicted in Fig. 1 for the single injection tracer study, and as in Fig. 2 for the primed tracer infusion study. All blood samples were drawn rapidly without stasis into dry plastic syringes through indwelling 19-gauge scalp vein needles which had been placed in an anticubital vein and which were maintained patent with physiological saline. Immediately following collection, a measured quantity of blood (20 ml) was dispelled from the syringe to a bottle containing water and barium hydroxide. Zinc sulfate was subsequently added to each bottle in order to precipitate proteins (4). The protein-free filtrates of blood were analyzed by enzymatic methods for both glucose (5) and lactate (6). Blood glucose specific activity was determined by liquid scintillation counting after ion-exchange separation from lactic acid (7). Lactate specific activity was determined from the dimedon derivative of acetaldehyde derived from lactate (8). Expired air from each subject was monitored continuously throughout each experiment with the aid of an automated on stream breath analyzer as described by Tolbert *et al.* (9).

Results. The results of a single injection study are shown in Fig. 1. Comparison of this curve with similar data published by Forbath *et al.* (1) indicates quite similar lactate kinetics in both man and dog. Employing standard methods of graphic analysis of the final slope of the lactate curve we have in fact obtained values very similar to those obtained by Forbath *et al.* for lactate turnover. Employing a curve integral analysis which utilizes all the data of the single tracer injection study (3), we have obtained values for lactate turnover in the resting human

[1] Obtained from International Chemicals, Nuclear Division, Irvine, CA. Diluted with pyrogen free solution and sterilized by Millipore filtration.

TABLE I. Lactic Acid Kinetics in the Human, Results of Single Injection vs. Continuous Infusion Studies.

	Single injection			Continuous infusion		
Subj.	T.R.[a]	R.R.[b]	Ox.R.[c]	T.R.	R.R.	Ox.R.
P.J.	149	5.1	146	128	4.0	130
G.E.	71	4.4	47	83	11.2	68
J.P.	75	10.1	48	75	10.4	50
E.R.	107.2	4.7	85	100	10.1	104
Avg	100.5	6.1	81.5	96.5	8.9	88

[a] T.R. = Lactic acid turnover rate. [b] R.R. = Reduction of lactate to glucose. [c] Ox.R. = Oxidation of lactate to CO_2. All values: mg/kg/hr.

subject that are listed in Table I along with corresponding values obtained by primed tracer infusion techniques in the same subjects. (The values listed for single injection studies are less than would be estimated by final slope analysis and compare well to values calculated from continuous infusion studies.) Also listed in this table are rates of reductive synthesis of glucose from lactate and rates of oxidation of lactate to CO_2. These values were computed from the data shown in Figs. 1 and 2 as previously described by Searle *et al.* (10). Note in Table I that there are random variations in all the indices listed: lactate turnover rate, rate of lactate reduction to glucose, and rate of lactate oxidation to carbon dioxide. Although the numbers of subjects studied is small, we are encouraged by the fact that the averages from both methods agree quite well and that by both techniques, the sum of the values for

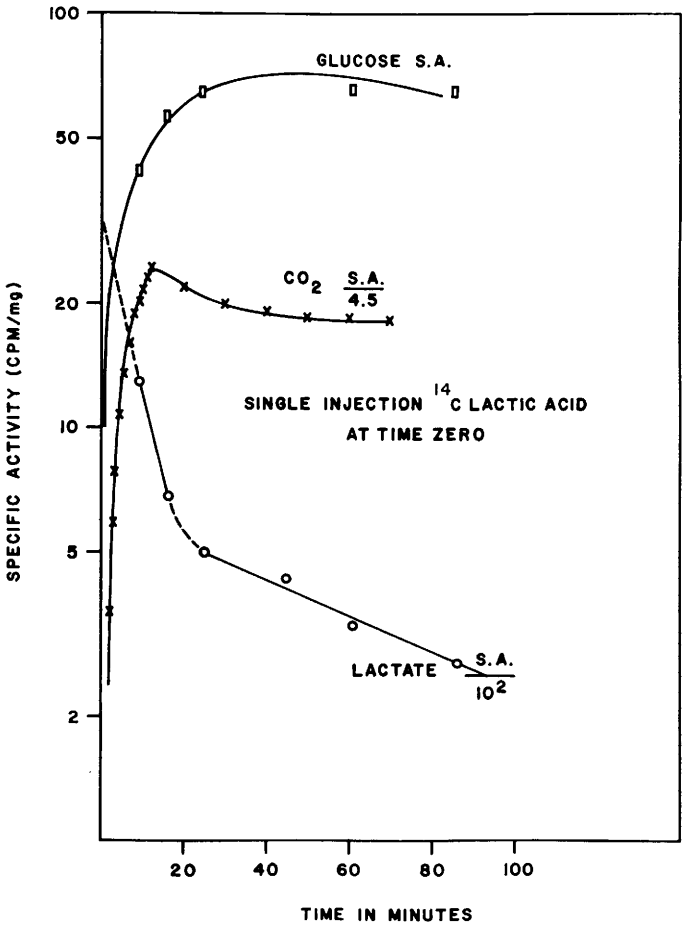

FIG. 1. Specific activity vs. time curve of lactate, glucose and expired CO_2 following a single tracer injection of 25 μCi of uniformly labeled ^{14}C-lactate.

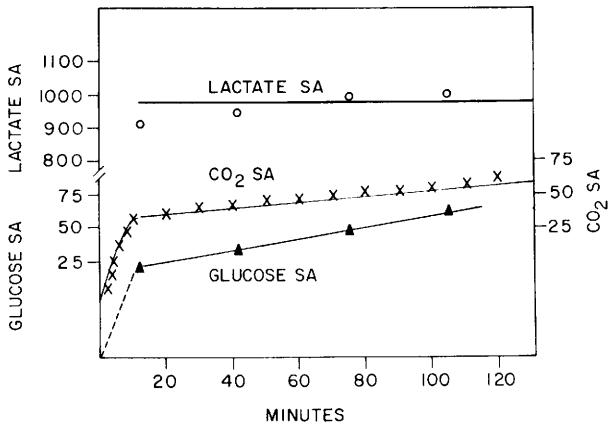

FIG. 2. Specific activity vs. time curve of lactate, glucose and expired CO_2 during the continuous infusion of 25 μCi of uniformly labeled ^{14}C-lactate.

reductive synthesis and for oxidation of lactate to CO_2, roughly equal the values for lactate turnover. From two data points taken early in primed infusion studies, estimates were made of lactate pool size (2). This information, together with lactate concentration and turnover rate, was used to produce estimates of virtual volume of distribution (space) of lactate and the turnover time of the lactate pool. These descriptive assessments of the body lactate pool are presented in Table II. A reassuring and, we feel significant, measure given in this table is the value for lactate space, expressed in % body wt. The average of these values is 49.4% of body wt. Although there are no ready references with which to compare this value of lactate space, it is reasonable to assume that a freely and rapidly diffusable molecule such as lactate would occupy a space at least equivalent to 3/4 of the body water space, as the above value indicates. Kreisberg *et al.* have arrived at similar fractional turnover rates for the lactate pool in humans starting with the assumption that lactate space would be equivalent to body water (11).

TABLE II. Lactic Acid in the Human—Pool Size, Space and Turnover Time. From continuous tracer infusion studies.

Subj.	Pool (g/kg)	Space (% body wt)	Turnover time (min)
P.J.	.035	52.5	20.0
G.E.	.028	43.2	15.5
J.P.	.023	57.0	19.0
E.R.	.032	45.6	18.4
Avg	.029	49.4	18.4

Discussion. Forbath *et al.* have rejected the single tracer injection method for estimation of lactate kinetics on the basis: that calculation of the rates of production of substances undergoing a very rapid turnover in the plasma from the terminal exponential component of the specific activity vs. time curve is subject to large errors. While this is so, it is certainly an insufficient basis for condemnation of the single-injection technique, for which computational procedures exist (3) that very adequately estimate production rates. Employing such procedures, we have demonstrated here that the single-injection technique yields values for lactate production in the human that are quite comparable to those obtained by the techniques of primed tracer infusion. In addition, we have observed that estimates of lactate reduction to glucose and oxidation to carbon dioxide are comparable for the two methods.

We suggest that the single-injection method for determining lactate turnover in humans, as we employ it, is superior to the infusion technique because it offers the following advantages : (a) The method is simple. (b) The data are obtained during a relatively short period (one hour). (c) Error due to recycling of label is relatively smaller

by the single-injection method than by the primed-infusion technique. The evidence presented here with respect to the magnitude of the various pathways of lactate metabolism is consistent with the findings of Drury and Wick (12) from studies in rabbits, and those of Annison *et al.* (13) from studies in sheep. Although they differ from those of Kreisberg *et al.* (14) in the human, with respect to CO_2 production from lactate, these authors admit that they have little confidence in their own measure of this index. In spite of this trepidation on their part, they have been led to speculate that since the lactate flux could not be completely accounted for in product pools, perhaps lactate flux was being overestimated due to a rapid equilibrium flux between lactate and pyruvate. We discount this argument on the basis of the ready accountability of carbon flux in the product pools in our study as well as in those of Drury (12) and Annison *et al.* (13). Indeed, we suggest that lactate kinetics in the human represent the flux (and perhaps total flux) of carbon through pyruvate. Using some preliminary figures we have developed for the flux of glucose to lactate (15), plus figures from past publications and other references which define glucose turnover and oxidation rates (16–18), and the figures presented in the present communication for lactate turnover and oxidation, we are able to derive approximately equal estimates of glucose oxidation given data from experiments employing either glucose or lactate as the administered labeled substrate. Thus, glucose turnover is approximately 100 mg/kg/hr with approximately 57 mg/kg/hr entering the lactate pool, lactate turnover is approximately 95 mg/kg/hr with approximately 90% being oxidized to CO_2. Note 90% of the 57 mg/kg/hr of glucose coming to lactate per hour is oxidized to CO_2. 90% times 57 = 51 mg/kg/hr which is the approximate rate of glucose oxidation derived from numerous studies with carbon-14 glucose (12–14).

In conclusion, we argue as do Steele (2) and Tait and Burnstein (3) that comparable information may be derived from single tracer injection and primed tracer infusion studies. Each has its own merits and shortcomings. The important fact that one should keep in mind is that whichever method is chosen, it does contain the basic kinetic information that one seeks from such experiments (if all the data are examined employing methods of mathematical analysis that are appropriate to the task at hand). With regard to the physiologic aspect of this study, we would draw the following conclusions: (a) Blood (body) lactate kinetics probably reflect the total flux of carbon through the metabolite pyruvate; (b) The primary fate of body lactate flux is not reduction to glucose in hepatic tissue, but oxidation to CO_2 probably in peripheral tissues as demonstrated by Drury *et al.* (19), and most recently in human forearm studies by Jorfeldt (20).

Summary. Comparison has been made between single injection and primed infusion methodology as a means of estimating turnover, oxidation, and reduction of lactate in the human. It is concluded that all these indices of lactate metabolism can be measured equally well with either the single injection or the primed infusion technique. The magnitude and fate of the lactate flux as determined in these studies suggest to us that lactate kinetics as measured with ^{14}C-lactate reflect the entire body flux of carbon through pyruvic acid.

This work has been supported in part by a grant from the U.S.V. Pharmaceutical Company, New York.

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Received Sept. 1, 1971. P.S.E.B.M., 1971, Vol. 139.