Diabetes, Insulin, Tolbutamide, and Glucose Load in the Degradation of C-14-labeled Lactate and Pyruvate

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SUMMARY

In two insulin-dependent diabetic patients, mild ketoacidosis was accompanied by a decrease in the formation of C-14-O_2 from DL-lactate-2-C-14 to about two thirds of the values obtained when the patients were in good control. One patient received the labeled compound by single intravenous injection in trace amount and the other by intravenous infusion with a load of DL-sodium lactate. Prior intravenous administration of insulin in these two patients caused no increase in output of C-14-O_2 above that in the control state, and a minor, insignificant increase in two milder diabetic patients (one given DL-lactate-2-C-14 with an infusion of glucose, the other DL-lactate-2-C-14 without glucose).

The activity of C-14 in expired carbon dioxide of diabetic patients (in good control) was 10 to 25 per cent less than that of one nondiabetic subject after administration of DL-lactate-2-C-14. In the latter patient, and in another nondiabetic given pyruvate-2-C-14, the rapid intravenous injection of 25 gm. of glucose twenty minutes before the labeled compound was accompanied by more rapid and extensive formation of C-14-O_2 than in the fasting state. Tolbutamide in two studies (one with DL-lactate-2-C-14 and a prior glucose load in a nondiabetic, and the other with DL-lactate-3-C-14 in a mild diabetic) had no apparent effect on the formation of C-14-O_2. Comparison of lactate-2-C-14 with lactate-3-C-14 in one diabetic patient showed 50 per cent higher specific activity of C-14-O_2 from the former labeled compound.

Studies of the concentration of lactic acid in the blood and rate of disappearance of DL-lactate-2-C-14 further indicated mild lactic acidemia which was associated with decreased elimination of lactic acid-C-14 from the blood of these diabetic patients.

The possibility that in diabetes there is a defect in utilization of intermediary carbohydrates, such as lactic and pyruvic acids, has not been extensively investigated. Pyruvic acid oxidation was found to be deficient in diaphragm[1,2] and cardiac muscle[2] of alloxan-diabetic rats, and was increased to some extent by addition of insulin in vitro.[1,2] Insulin in the dog in vivo enhances the net hepatic uptake of both pyruvic and lactic acids.[3] The plasma concentration of these compounds is increased in diabetic patients,[4] but this abnormality is considered to be most pronounced in diabetes associated with excess glucocorticoids.[5] Recent recognition of the syndrome of lactic acidosis,[6] which may develop in diabetics under some circumstances,[7] provides new cause for further investigation of the nature and degree of abnormality of metabolism of lactic acid in diabetes. This paper reports findings on the disappearance of C-14-labeled lactic acid from the blood of patients with varying degrees of, or no, diabetes and the concomitant appearance of C-14 in the expired carbon dioxide. The effect on lactic acid oxidation of administration of insulin (with or without glucose) or tolbutamide to the diabetic has been tested, and also the effect of glucose alone on oxidation of lactic or pyruvic acid in the nondiabetic has been determined.

EXPERIMENTAL SUBJECTS

Seventeen studies were conducted with four diabetic and two nondiabetic subjects. Some fundamental statistics about these patients are contained in table 1, and more information about the clinical conditions pertaining to various studies have been published earlier.[8] In the "uncontrolled" diabetes of both R.P. and J.D. there were heavy glycosuria and ketonuria. Clinical symptoms of acidosis were present, although there was only slight to moderate decrease in plasma CO_2 concentration.[8] Blood pressure readings did not suggest any peripheral circulatory collapse, nor was there any obvious hyperventilation.

All patients were studied after an overnight fast. Subcutaneous injections of medium-acting insulin were not given within twenty-four hours before study (except for J.D. in the controlled and insulin studies, vide infra) nor of unmodified insulin within twelve hours. Measurement of rate of output of total CO_2 (table 1) indicated that during all studies patients were in a comparable, and close to a basal, resting state. Repeated studies in patients were separated by at least one month.

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TABLE 1

General clinical and experimental data on patients

Subject	M.B.	P. V.	V. K.	C. S.	R. P.	J. D.
Age	32	37	44	60	44	32
Sex	F	F	M	M	F	F
Height (cm.)	163	179	166	163	150	163
Weight (kg.)	49-53	63	74-78	62	52-57	56
Condition	nondiabetic (cyclic edema)	nondiabetic (functional hypoglycemia)	mild diabetic	mild diabetic	moderately severe diabetic	severe juvenile diabetic
Duration of diabetes (yrs.)			4	40	0-.7	26
Therapy			tolbutamide	tolbutamide	insulin	insulin
Average rate of output of C-12-O_2 (mM/min.)	7	6½	8	8	6	6½

MATERIALS AND METHODS

Glucagon-free insulin in a dose of 0.1 U. per kilogram body weight was either injected five to ten minutes before intravenous injection of the labeled compound or was infused thirty to forty-five minutes prior to, and forty-five to sixty minutes after, the labeled compound. Twenty-five grams of glucose in 50 per cent solution were injected over a four-minute period approximately twenty minutes before injection of the labeled compound or else provided in the same dose as an infusion with insulin. Tolbutamide, 0.04 gm. per kilogram, was given intravenously fifteen to twenty minutes before the C-14 compound.

The source, purification, standardization, preparation, specific activity, and dose of the C-14-labeled lactic and pyruvic acids have been previously described.[8] Except for the studies with R.P., in which the DL-lactic acid-2-C-14 was added to 500 ml. of 1/6 M sodium lactate for intravenous infusion over sixty minutes (Bowman pump), all labeled compounds in 10 to 25 ml. of 0.9 per cent NaCl were injected in one to two minutes into an antecubital vein.

Breath samples of one to two minutes' duration were collected into rubber balloons[9,10] at timed periods after injection, usually 15, 30, 45, 60, 90, 120, 180, 240 and 360 min. Generally within sixty minutes the gas in the balloons had been percolated through sintered-glass plates into 1 to 2 N NaOH solution. Known aliquots of the solutions were assayed by the Van Slyke gasometric apparatus for content of CO_2, and the latter gas transferred to Bernstein-Ballentine tubes for proportional counting.[11] Samples were repeatedly analyzed until standard error was less than 3 per cent.

The C-14-O_2 and C-12-O_2 in the breath were further measured in some cases by prolonged (15 to 60 min.) continuous passage through an ionization chamber and infrared spectrometer with continuous recording of functions for concentration of these gases.[12] A measure of the CO_2 rate output was obtained by count of total gas flow with a wet-test gas meter together with the data from the infrared analysis.[12] These records were particularly useful in establishing shapes and peak times of specific activity curves as well as providing a crosscheck with the gas proportional counting.

Concentration of lactic acid in a trichloracetic acid filtrate of blood was determined by the Barker-Summerson method.[13] For the analysis of lactic acid-C-14 in the blood either the bulk of the TCA filtrate or the alkaline eluate of anion resin columns used in purification of glucose[8] were available. In most cases both were assayed. To the filtrate or acidified eluate were added $MnSO_4$ and $KMnO_4$ to form acetaldehyde, which was aerated into buffered dimedon solution.[14] The fine crystalline precipitate of acetal-dimedon was filtered, weighed, and transferred to vials containing liquid scintillation medium for determination of specific activity with a Packard Tri-Carb spectrometer. The assay was standardized with DL-lactic acid-2-C-14 which had been measured by combustion.[11]

RESULTS

Figure 1 shows the time curves of specific C-14 activity of expired carbon dioxide in the breath of the juvenile-type diabetic patient, J.D., after single injection of a trace amount of DL-lactate-2-C-14 in each of three clinically contrasting conditions. In the "controlled" state the patient had last received 10 U. NPH Insulin twelve hours before study. At the time of study she had fasting blood sugar concentration of 245 mg. per 100 ml., 3+ glycosuria, no ketonuria, and no symptoms of

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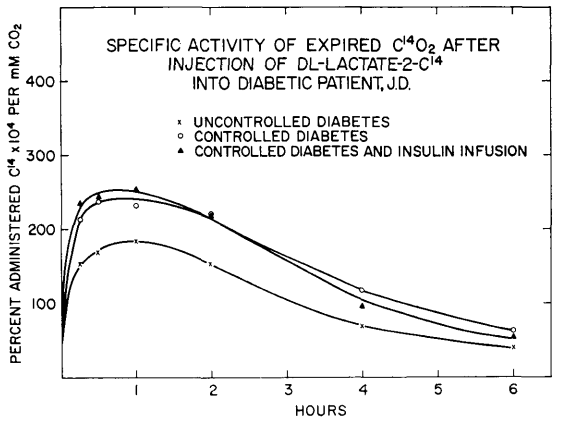

FIGURE 1

acidosis. In the insulin study the patient was well controlled at the time of intravenous insulin injection (blood sugar = 109 mg. per 100 ml., no glycosuria, no ketonuria) and the level of blood glucose fell to 56 mg. per 100 ml. at forty-five minutes after injection of lactate-C-14. There was evidently no significant effect of insulin on the rate of oxidation of the lactate-C-14. However, when the patient on a third occasion was studied in a clearly uncontrolled state which was characterized by fasting blood sugar of 360 mg./100 ml., 3+ glycosuria, 4+ ketonuria, and signs and symptoms of acidosis,[8] the output of C-14-O_2 was significantly lower than in the other two conditions.

This same pattern of excretion of labeled carbon dioxide was observed in another series of studies with the diabetic patient, R.P., (figure 2), who received the DL-lactate-2-C-14 somewhat differently; i.e., in an infusion with a load of DL-sodium lactate. In this case the study in the "uncontrolled" state was done within two days after recognition of development of florid diabetes and while mild ketoacidosis was present.[8] The excretion of

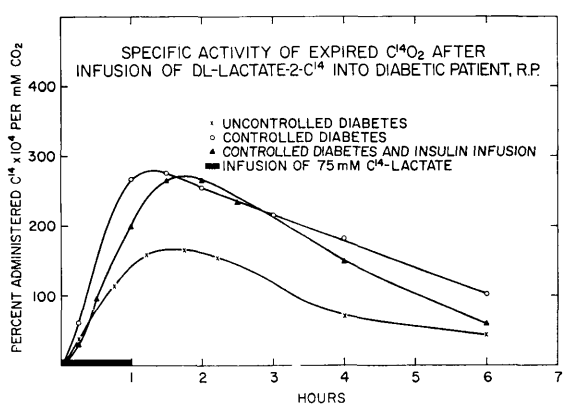

FIGURE 2

labeled carbon dioxide was evidently much lower than on subsequent occasions when the patient was well controlled with daily insulin or furthermore received prior intravenous infusion of insulin.

The infusion of the C-14 compound with a load of sodium lactate in R.P. was intended to obliterate differences among the three studies in plasma concentration of lactic acid, which in itself might affect the rate of utilization and oxidation of the labeled lactate. Measurement of the lactic acid concentration as well as rate of disappearance of lactate-C-14 from plasma (figure 3) indicated approximately equal concentrations of total lactic acid at the end of the infusion but delayed subsequent disappearance of the (labeled and unlabeled) lactate in the "uncontrolled" state. The question arises whether the delayed utilization could account for the decreased formation of C-14-O_2. Using the rather rough measure of 6 mM CO_2 per minute in the breath (table 1) and estimating the miscible body bicarbonate pool as 12 mM/kg. body weight,[9,15] then the per cent of infused lactate-C-14 converted to CO_2 by the end of two hours after start of infusion was ca. 19 per cent in the "uncontrolled" state and ca. 33 per cent in the other two studies. If the unutilized lactate-C-14 were contained in the extracellular fluid space at a concentration uniformly

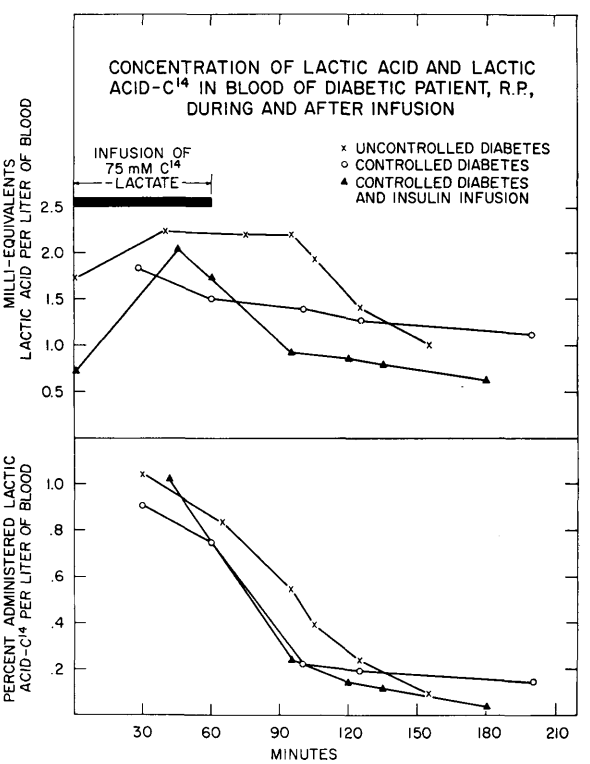

FIGURE 3

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that of plasma, then the corresponding amounts of unutilized lactate-C-14 would be 3 per cent and 1.5 per cent of the infused amount in the "uncontrolled" and "controlled" states, respectively. Evidently delay in transfer of lactate from the extracellular fluid space does not adequately account for the decreased appearance of C-14-O_2.

Some further comparisons of disappearance of labeled lactic acid from the blood are shown in figure 4. Both the juvenile diabetic, J.D., in the "uncontrolled" state and a mild diabetic, C.S., showed similar blood concentrations of total lactic acid, which were about twice the normal value, as exemplified with the nondiabetic subject, M.B. However, the disappearance of labeled lactic acid from the blood was much slower in the case of the more severely diabetic patient, which suggests a slowed turnover of blood lactic acid. Differences in pool size (intra- as well as extracellular) or metabolic recycling of C-14 could also account for the disparities between these two patients.

The specific activity of the carbon dioxide-C-14 in the breath of C.S. is shown in table 2. The output in the

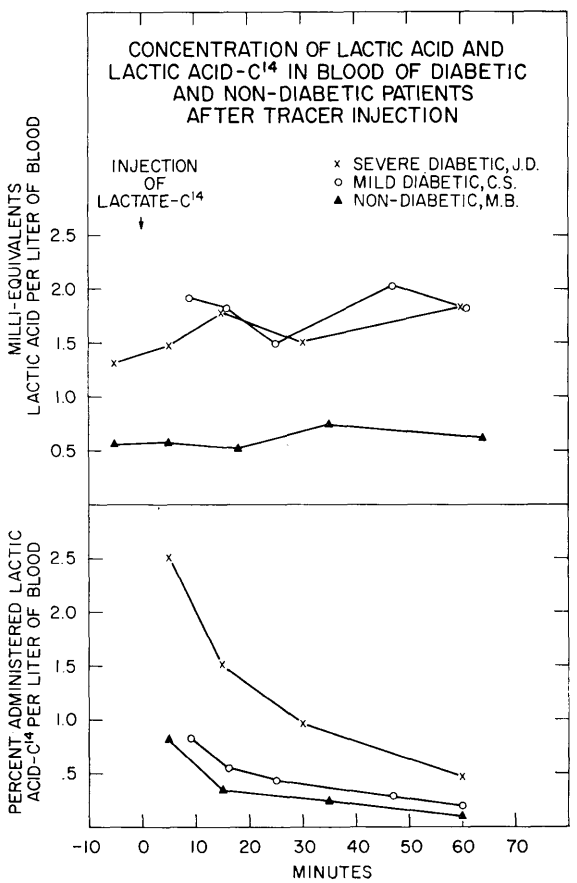

FIGURE 4

fasting state was obviously higher than for J.D. in the "uncontrolled" state. Again the differences (30 per cent *vs* 19 per cent of the dose in the breath and body bicarbonate at one hour after injection) can not be accounted for by the amounts of unutilized lactate-C-14 in the extracellular fluid space (2.5 per cent and 5 per cent for C.S. and J.D., respectively). Table 2 also shows results of another study of C.S. in which insulin, glucose, and sodium bicarbonate were infused before and during the utilization of lactic acid-2-C-14. There was a slight (about 10 per cent) increase in output of C-14-O_2 compared with the fasting state in C.S. which in general confirms the observations with J.D. and R.P. that exogenous insulin has no significant effect.

Table 2 further shows the activities of C-14-O_2 in the breath of another mild diabetic subject, V.K., who on various occasions was given DL-lactate-3-C-14 as well as the 2-labeled compound. The sparse data from a study with insulin injection and lactate-3-C-14 suggest again a slight but not notable increase in conversion to C-14-O_2 compared with the fasting state. In a study in which tolbutamide was injected intravenously prior to lactate-3-C-14 there was also no significant effect on the output of C-14-O_2. In all three of the latter studies there was a slower and lesser output of C-14-O_2 than in a fourth study in the fasting, untreated state with DL-lactate-2-C-14. This would be expected if an appreciable fraction of pyruvic acid deriving from lactate were oxidatively decarboxylated and introduced into the TCA cycle as an acetyl derivative rather than through carboxylation to malate or oxaloacetate.[10]

Figure 5 shows results of studies in two nondiabetic subjects in the fasting state and after an acute intravenous glucose load. One of these patients (P.V.) received pyruvic acid-2-C-14 instead of DL-lactic-acid-2-C-14. In the fasting state these two compounds appear to be oxidized to about the same extent. In either case when there is an acute intravenous glucose load given about twenty minutes before the labeled compound the C-14 appears in the expired carbon dioxide much more quickly and also more extensively. This was more pronounced with pyruvic acid than with lactic acid. In a third study with M.B. (not shown), in which both a glucose load and tolbutamide were given intravenously before the lactic acid-C-14, the output of C-14-O_2 in the breath was approximately the same as with glucose load alone.

The peak activity of C-14-O_2 for M.B. in the fasting state was 10 to 25 per cent higher than the range for the mild or well controlled diabetic patients also given

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TABLE 2

Specific activity (per cent of administered C-14 x 10^4 per mM CO_2) of expired C-14-O_2 after injection of DL-lactate-2- or 3-C-14 in diabetic patients

Patient	Condition	C-14-Compound injected	Blood glucose mg./100 ml.	Minutes after C-14 injection 15	30	60	90	120	240
V. K.	fasting	DL-Lactate-3-C-14	130	120	141	151	151	149	96
	fasting and tolbutamide	DL-Lactate-3-C-14	148-80	92	118	151		163	89
	fasting and insulin	DL-Lactate-3-C-14	140-70	152				175	92
C. S.	fasting	DL-Lactate-2-C-14	125	215	220	215	209	187	78
	fasting	DL-Lactate-2-C-14	200	256	269	264	247	228	122
	glucose and insulin infusion	DL-Lactate-2-C-14	233-390	227	272	285	288	259	195

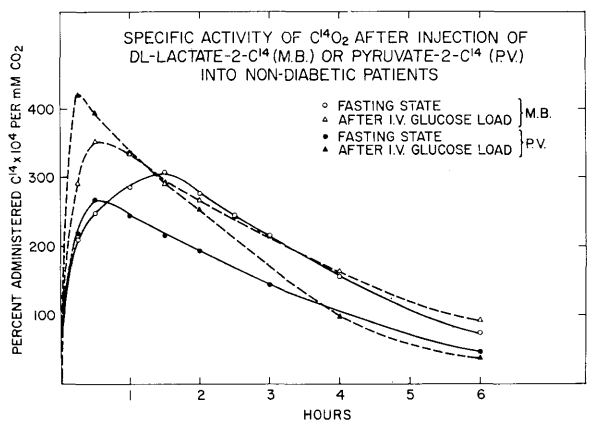

FIGURE 5

single injection of DL-lactate-2-C-14, but further cases are needed to establish any significant difference in this regard.

DISCUSSION

These studies suggest that the administration of insulin in vivo does not promote the utilization and oxidation of lactic acid in any way comparable to its direct and profound effect on the utilization of glucose. However, in the diabetic patient who is so deficient in exogenous or endogenous insulin that a state of ketoacidosis of even mild degree exists, then an impairment in the rate of disappearance of labeled lactic acid from the blood and oxidation to carbon dioxide can be demonstrated. Similar studies by McManus et al.[16] have also indicated such a defect in the oxidation of pyruvate-2-C-14 by ketotic diabetics. Although a delay in utilization of lactate or pyruvate may be in some way attributable to an absolute deficiency of insulin per se, it seems more probable that a relative or absolute excess of 11, 17-oxygenated adrenal steroids and/or growth hormone is responsible for the abnormality noted by us and by McManus et al. in diabetic patients. Both the adrenal steroids in humans[5] and growth hormone in dogs[17] have been shown to increase the blood levels of pyruvic acid which was interpreted as a probable impairment in utilization.

Since the conversion of C-14 to blood glucose was measured in these same patients and generally found to be higher in diabetics than nondiabetics and lower after insulin or tolbutamide administration,[8] it could be supposed that a branch-point of alternate metabolic pathways for lactate, pyruvate, or subsequent metabolites is affected in diabetes. This could occur after the formation of dicarboxylic acids of the TCA cycle, which are intermediates in the pathways both to CO_2 and glucose.[18] However, a more careful comparison of the relative changes in conversion to carbon dioxide and to glucose in the individual studies in these patients[8] suggests that the two abnormalities may be unassociated to some extent, since insulin appears to cause more significantly a decrease in conversion to glucose, while a decrease in conversion to carbon dioxide is more typically characteristic of gross lack of control of diabetes.

Relatively mild or even subclinical diabetic states could be accompanied by depressed oxidation of lactate or pyruvate if the diabetes were secondary to cortical steroid or growth hormone excess. More recent studies in our laboratory (to be fully reported later) indicate that mild or subclinical diabetes in the obese type of patient may be associated with a decreased formation of C-14-O_2 from these labeled compounds.[19] Further information about hormonal effects on turnover and size of successive substrate pools is required for an understanding of the nature and location of metabolic abnormality

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suggested by such findings.

The mild lactic acidemia found in two diabetic patients (one severe, one mild) is further evidence of some impairment of utilization at this level of carbohydrate metabolism, as suggested also by another recent finding in a larger group of diabetic patients.[4] However, even in the severe diabetic the lactic acidemia was not nearly of the magnitude found in some cases of lactic acidosis causing acute symptoms in diabetic patients.[7] Evidently there were special circumstances in the histories of the latter patients which besides diabetes predisposed to an accumulation of excess lactate. The latter condition can apparently occur in a variety of chronically ill patients.[6]

An interesting finding was the marked increase in rate of oxidation of lactate-2-C-14 and pyruvate-2-C-14 caused by prior intravenous glucose load in the two nondiabetic subjects. One could expect a decrease in conversion to C-14-O_2 on the basis of (1) simple pool dilution of the labeled substrate by unlabeled 3-carbon intermediates from glycolysis, and (2) diversion of 2-carbon derivatives of pyruvate to formation of fatty acids rather than CO_2 by virtue of the lipogenic effect of glucose utilization.[20] Various theories might explain the contrary finding. A glucose load (aided perhaps by insulin released from the pancreas or unbound from protein) may curtail the flow and utilization of free fatty acids, thereby decreasing the substrate dilution of intermediates between the labeled 3-carbon compounds and carbon dioxide. There may be changes in activities of coenzymes or intracellular concentrations of substrates which cause relative increase in utilization of pyruvate by oxidative decarboxylation compared with carboxylation to intermediates of the tricarboxylic acid (TCA) cycle. Freedman and Graff[21] found that prior feeding of rats changes the distribution of C-14 in glutamic acid formed from DL-alanine-2-C-14 in such a way as to suggest diversion to the pathway of oxidative decarboxylation. Studies with C-14-labeled acetate[10] indicate that this diversion would accelerate appearance in carbon dioxide of C-14 from pyruvate- or lactate-2-C-14.

The observation that DL-lactate-2-C-14 appears in the expired carbon dioxide about 50 per cent faster than DL-lactate-3-C-14 is similar to another finding with rat liver slices[20] and indicates that some of the intermediate pyruvate proceeds to CO_2 via decarboxylation to acetyl derivative. On the other hand, an appreciable fraction may be entering the TCA cycle via carboxylation, since if all of the oxidized lactate took the pathway of decarboxylation, the carbon from the 2 position of lactate would provide 100 per cent higher specific activity in carbon dioxide than the carbon from the 3 position according to results with the corresponding pair of labeled acetate compounds.[10]

The present findings do not permit estimations of values for turnover in the blood or rate or extent of oxidation of natural lactic acid because of the use of the racemic labeled compound. It has been shown by Sachs[22] that in humans the natural L-lactic acid-1-C-14 is converted four times as fast to carbon dioxide as D-lactic acid-1-C-14. Our results are nevertheless in qualitative agreement with the observation of Williamson[23] that insulin does not directly increase the utilization of L-lactic acid by the perfused rat heart.

SUMMARIO IN INTERLINGUA

Diabete, Insulina, Tolbutamida, e Cargation de Glucosa in le Degradation de Lactato e Pyruvato Marcate con C-14

In duo diabeticos in dependentia de insulina, leve grados de ceto-acidosis esseva accompaniate de un declino in le formation de C-14-O_2 ab DL-lactato-2-C-14 ad circa duo tertios del valores obtenite quando le patientes esseva ben stabilisate. Un del patientes recipeva le marcate composito per un sol injection intravenose in un oligo-quantitate, le altere per infusion intravenose con un carga de DL-lactato de natrium. Le antecedente administration intravenose de insulina in iste duo patientes causava nulle augmento del rendimento de C-14-O_2 in supra del valores determinate in le stato de controlo. Illo causava un minor, statisticamente non significative augmento in duo altere minus severmente afficite diabeticos qui esseva tractate le un con DL-lactato-2-C-14 con un infusion de glucosa, le altere con DL-lactato-2-C-14 sin glucosa.

Le activitate de C-14 in le expirate bioxydo de carbon de patientes diabetic in stato de bon stabilisation esseva 10 a 25 pro cento minus que illo de un subjecto sin diabete post le administration de DL-lactato-2-C-14. In iste ultime patiente, e in un altere non-diabetico qui recipeva pyruvato-2-C-14, le rapide injection intravenose de 25 g de glucosa vinti minutas ante le marcate composito esseva accompaniate de un plus rapide e plus extense formation de C-14-O_2 que in stato jejun. Tolbutamida—in un studio con DL-lactato-2-C-14 post carga de glucosa in un subjecto non-diabetic e in un altere studio con DL-lactato-3-C-14 in un patiente con leve diabete—habeva nulle apparente effecto super le formation de C-14-O_2. Le comparation de lactato-2-C-14 con lactato-3-C-14 in un patiente diabetic monstrava un 50 pro cento plus alte activitate specific de C-14-O_2

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in le caso de lactato-2-C-14.

Studios del concentration de acido lactic in le sanguine e le rapiditate del disparation de DL-lactato-2-C-14 indicava leve grados de acidemia lactic que esseva associate con un reducite elimination de lactic acido-C-14 ab le sanguine de iste patientes con diabete.

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