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CONVERSION OF DL-LACTATE-2-C^{14} OR -3-C^{14} OR PYRUVATE-2-C^{14} TO BLOOD GLUCOSE IN HUMANS: EFFECTS OF DIABETES, INSULIN, TOLBUTAMIDE, AND GLUCOSE LOAD *

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Much investigation in recent years has centered on the phenomenon of hepatic overproduction of glucose in diabetes and particularly the question as to whether insulin acts significantly to decrease the overproduction. Measurements of hepatic glucose balance by multiple cannulation *in situ* with varying degrees of physiological disturbance have led to conflicting findings. Other studies with singly or continuously administered C^{14}-glucose have been variously interpreted as to the timing, extent, or mode of action of insulin, tolbutamide, or glucose load on hepatic glucose output. The subject has been reviewed recently by Cameron (1).

A different approach to this question has employed the measurement of incorporation *in vivo* of C^{14} from labeled metabolic intermediates into glucose or liver glycogen in animals (2–4) and glucose in man (5, 6). For further and more specific study in this direction, diabetic and nondiabetic patients have been given intravenous injections of trace amounts of C^{14}-labeled lactate or pyruvate followed by serial analysis of blood glucose for C^{14} content. Subsequently, glucagon was injected in an attempt to estimate relative glycogen labeling also. The effects of insulin, tolbutamide, and glucose load have been studied in the same patients.

EXPERIMENTAL SUBJECTS

Seven diabetic and three nondiabetic subjects are reported in this study. Table I lists age, sex, height, weight, duration of diabetes, type of therapy, and diet before study. The more severe diabetics O.S. and J.D. showed 4+ glycosuria and 4+ ketonuria at the time of study in each case. W.G. had 3+ glycosuria and 2+ ketonuria in study I and only a trace of glycosuria in study II. Other patients (except R.P.; see below) had no glycosuria or ketonuria at the time of study.

In the only study with O.S., her plasma CO_2 at the start was 21 mEq per L and 2 hours later was 18 mEq per L. In study III in J.D. (with tolbutamide), plasma CO_2 was 25 mEq per L at the beginning and 21 mEq per L at the end. W.G. and K.L. had weak, delayed hypoglycemic response to iv tolbutamide. A.B. and V.K. showed the more typical hypoglycemic response of mild diabetics to iv tolbutamide. M.B. (with cyclic edema) showed normal oral glucose tolerance curves by single 100-g dose as well as by divided dose (7). E.B. (with polycythemia vera) and J.L. (with postmyocardial infarction) both had borderline rates of glucose disappearance after rapid iv injection of 25 g.

Study I in R.P. was conducted within 2 days after diagnosis of diabetes and before any therapy other than bed rest and fluids by mouth. There had been classical onset of progressive fatigue, polyuria, polydipsia, and weight loss for 6 months. At the time of study I, marked glycosuria and ketonuria were present, and plasma CO_2 was 27 mEq per L. Study II occurred 4 months later, after the patient had been well controlled with insulin and had gained 5 kg. No glycosuria or acetonuria was present on admission or after insulin was withheld for 2 days before study II. Study III was conducted under the same circumstances as study II except that no insulin was infused. C^{14}-lactate in all studies with R.P. was contained in 500 ml of 1/6 M iv sodium lactate given during 1 hour.

All patients were studied in a resting condition in the morning after an overnight fast. In the case of all diabetic patients, medium-acting insulin [1] was not given within 24 hours before study nor unmodified insulin [2] within 12 hours.

MATERIALS AND METHODS

Glucagon-free insulin,[3] 0.1 U per kg body weight, was given by rapid iv injection 5 to 10 minutes before iv injection of the labeled compound, except in study II in R.P. in which 4 U in 0.9% sodium chloride was infused at a uniform rate during 90 minutes, starting 30 min-

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[1] NPH Iletin, Eli Lilly & Company, Indianapolis, Ind.
[2] Regular Iletin, Eli Lilly & Company, Indianapolis, Ind.
[3] Courtesy of Eli Lilly & Company, Indianapolis, Ind.

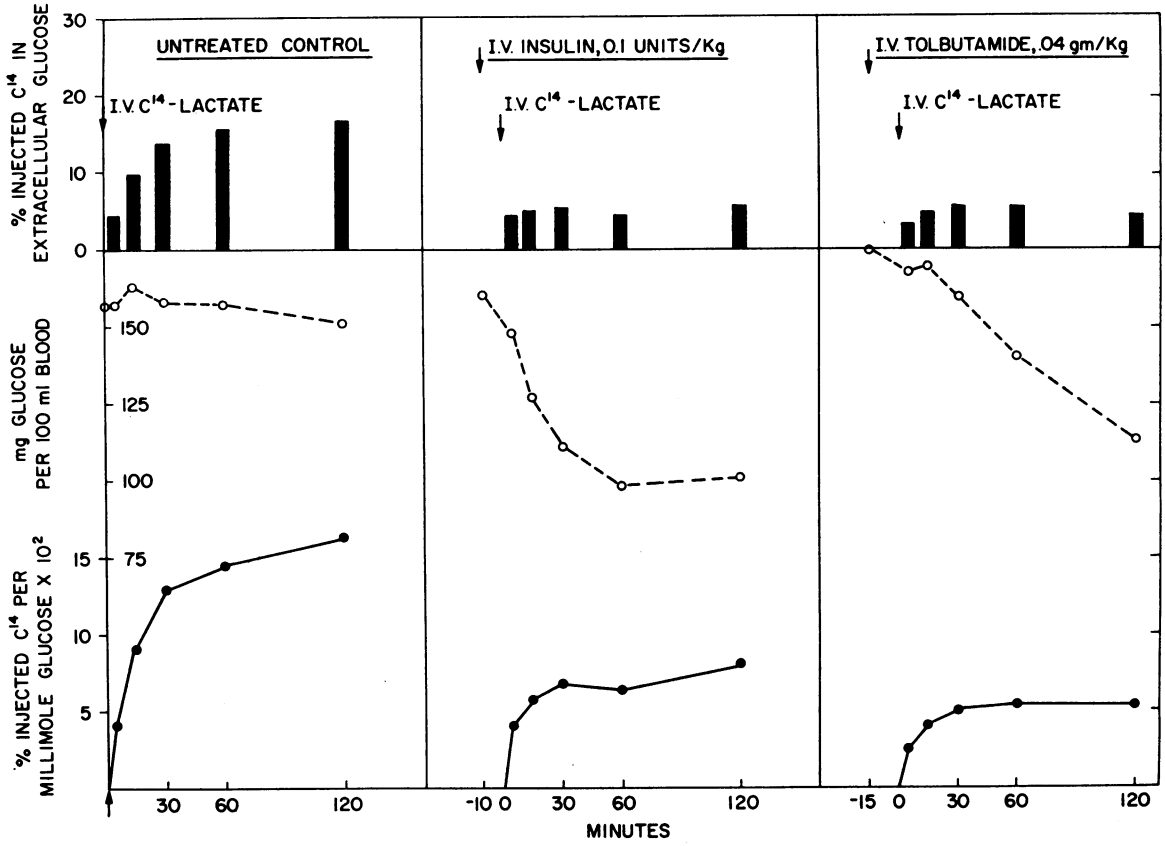

FIG. 1. GLUCOSE FORMATION FROM DL-LACTATE-3-C^{14} IN MILD DIABETIC PATIENT A.B.

utes before C^{14}-lactate infusion. Tolbutamide,[4] 0.04 g iv per kg, was given 15 to 20 minutes before the C^{14} compound. Glucagon,[5] 2 mg im (iv in study I in R.P.), was given approximately 2 hours after injection of a labeled compound.

The radioactive substances DL-zinc lactate-2-C^{14} and sodium pyruvate-2-C^{14} were obtained commercially.[6] The pyruvic acid was passed through a Dowex 1 anion exchange column (chloride form) with gradient elution by 0.1 N HCl solution. The pyruvic acid fraction was identified by radioactivity measurements and by fluorescence identification (8). Zinc lactate was prepared for use by carrier addition, then passage through a cation exchange column of Dowex 50 (200–400 mesh). Both lactic and pyruvic acid solutions were sterilized by filtration through ultrafine sintered glass after addition of sodium chloride to isotonicity. Doses of 40 to 150 μc C^{14} were administered in single injections contained in 0.5 to 0.8 mmoles for lactic acid and 0.05 to 0.25 mmoles for pyruvic acid. Repeated studies in patients were spaced at least 1 month apart.

Blood samples of 50 to 100 ml were collected from an antecubital vein of the arm opposite to that used for injection at intervals usually of 15, 30, 60, 120, and 180 minutes after C^{14} injection. Blood was immediately pooled with 5 vol water, and protein-free filtrates were prepared within 15 to 30 minutes. Filtrates were passed successively through cation and anion resin exchange columns (2). Eluates were evaporated to small volume *in vacuo,* and phenylglucosazones were prepared without addition of unlabeled glucose (2). After two precipitations of the osazone, specific activities did not change. Osazones were oxidized, and CO_2 was measured by Van Slyke techniques, including transfer to Bernstein-Ballentine tubes for proportional counting of gaseous $C^{14}O_2$ (9). Likewise, samples of injected solutions were oxidized and counted after appropriate dilution with unlabeled lactate or pyruvate. Combustion of all samples was repeated 2 to 4 times or more until standard deviation of count among samples was less than 5%.

RESULTS

Figure 1 illustrates the general finding that single injections of insulin and more clearly, tol-

[4] 1-Butyl-3-(*p*-tolylsulfonyl)urea; Orinase sodium, courtesy of The Upjohn Company, Kalamazoo, Mich.

[5] Crystalline, hydrochloride; Eli Lilly & Company, Indianapolis, Ind.

[6] Volk Radiochemical Co.

TABLE I

General data on experimental subjects

Subject	O.S.	J.D.	W.G.	R.P.	K.L.	A.B.	V.K.	M.B.	E.B.	J.L.
Age, *years*	53	32	43	44	70	63	44	32	56	34
Sex	F	F	M	F	F	F	M	F	M	M
Height, *centimeters*	161	163	173	150	152	160	166	163	156	174
Weight, *kilograms*	67	56	107	52–57	56	60	76	49	65	84
Duration of diabetes, *years*	12	26	2	0–.7	3	7	4			
Therapy	Insulin	Insulin	Tolbutamide	Insulin	Diet	Tolbutamide	Tolbutamide			
Total daily diet, *kilocalories*	1,725	1,800	1,650	2,000	1,800	1,500	1,800	2,500	2,500	1,800
Carbohydrate, *grams*	190	200	187	222	172	195	273	240	290	215
Protein, *grams*	72	70	89	99	76	72	89	90	90	70
Fat, *grams*	80	90	59	79	89	50	40	130	110	73

butamide reduce the apparent incorporation of C^{14} from labeled lactate into the mass of miscible free glucose. In other studies in man (10–12), the latter has been estimated to be distributed in a hypothetical pool at blood concentration with a volume of about 20% body weight, i.e., roughly equivalent to extracellular space (11). Some estimates in man have suggested 25% (13) and 30% (14) body weight. The work of Steele, Wall, De Bodo, and Altszuler (15) with dogs indicates two major pools of glucose, one equilibrating rapidly, in 20 minutes, within about 13% of body weight, and another after about 60 minutes to a total pool twice as large. The data of Segal, Berman, and Blair (12) suggest similar, but somewhat smaller, glucose pools in man. According to Hetenyi, Wrenshall, and Best (16), insulin or insulin and glucose increase the apparent glucose space about 50% of the preinsulin value within the first 30 minutes and 100% after 60 minutes in the normal, but not in the depancreatized dog, despite a blood sugar-lowering effect of insulin. The effect of hypoglycemic agents on the size of glucose "space" in diabetic man is not known. The calculation of extracellular glucose-C^{14}, however, as defined by the product of blood glucose concentration, specific radioactivity of blood glucose, and 20% body weight, presently appears to be the best average estimate of the amount of glucose-C^{14} present at any time after the first 15 or 30 minutes. Besides a possible increase in glucose space after insulin, consideration should be given to the amount of glucose-C^{14} that is utilized after insulin in excess of the basic utilization during the control state. Using the net fall in blood sugar level and the specific radioactivity of glucose in successive time intervals, we have calculated that at most this factor does not add more than 4% of the injected C^{14} to the calculated formed glucose in any of the studies.

Figure 1 and data from the other study with lactate-3-C^{14} (Table II) indicate that the reduction in extracellular glucose-C^{14} after tolbutamide occurs before there is any appreciable fall in blood glucose level, and thus before any general disturbance in glucose homeostasis. On the other hand, when hypoglycemia follows administration of insulin, as in some of our studies, glycogenolytic response in the liver to adrenalin secretion (1, 17) may lower the peripheral glucose-C^{14} specific activity due to intrahepatic dilution of newly formed glucose-C^{14}.

Figure 2 shows the results of three studies in a diabetic patient given DL-lactate-2-C^{14} in the iv infusion of a lactate load. This method of administration was used in order to erase possible differences in lactate pool size between the contrasting states of no treatment and insulin administration (18). In all three studies, blood levels of lactate [7] were between 16 and 20 mg per 100 ml during infusion, although the level remained relatively high for some time after infusion in the acutely diabetic condition. By conversion of lactic acid to an acetaldehyde-dimedone [8] derivative for radioassay, it has been found [9] that the specific activities of lactic acid in whole blood at about the midpoint of infusion were also very similar in relation to dose injected, differing by less than 10% among the three studies.

The decrease in the extracellular glucose-C^{14} after insulin was greater with patient R.P. (Fig-

[7] Barker-Summerson method (19).

[8] 5,5-Dimethyl-1,3-cyclohexanedione.

[9] Unpublished observations of Dr. Y. Shigeta.

TABLE II

Blood glucose concentration and calculated extracellular glucose-C^{14} of four patients in various experimental conditions after intravenous administration of C^{14}-labeled lactate

Subject		V.K.			W.G.		J.D.			M.B.		
Disease, state Labeled compound		Mild diabetic DL-lactate-3-C^{14}			Mild diabetic, obese DL-lactate-2-C^{14}		Insulin-dependent diabetic DL-lactate-2-C^{14}			Nondiabetic, cyclic edema DL-lactate-2-C^{14}		
Treatment*		Control	Insulin	Tolbut-amide	Control	Insulin	Control	Insulin	Tolbut-amide	Control	Glucose load	Glucose load and tolbutamide
Time, *minutes*	−15	137	141	147	270	224	355	346	333	88	210	165
Glucose, *milligrams per 100 ml blood*	15	142	76	129	269	185	378	306	355	88	167	86
	30	141	71	121	274	169	382	287	358	90	137	63
	60	135	80	112	254	148	379	262	368	91	114	42
	120	127	99	80	258	158	356	247	375	91	75	67
	180†									187	139	125
Injected C^{14} in extra cellular glucose, %	15	18	9	8	24	11	10	12	20	10	8	1.5
	30	24	6	9	28	14	20	16	25	14	8	1.5
Time, *minutes*	60	24	9	8	28	13	24	12	29	14	7	2
	120	19	11	6	26	11	24	14	21	11	6	4
	180†									9	11	3

* Control state = fasting without treatment; insulin treatment = 0.1 U iv per kg body weight given at − 5 to − 10 minutes; tolbutamide treatment = 0.04 mg iv per kg at − 15 to − 20 minutes; and glucose load = 0.33 g iv glucose per kg at − 20 minutes.

† Fifty minutes after 2 mg im glucagon.

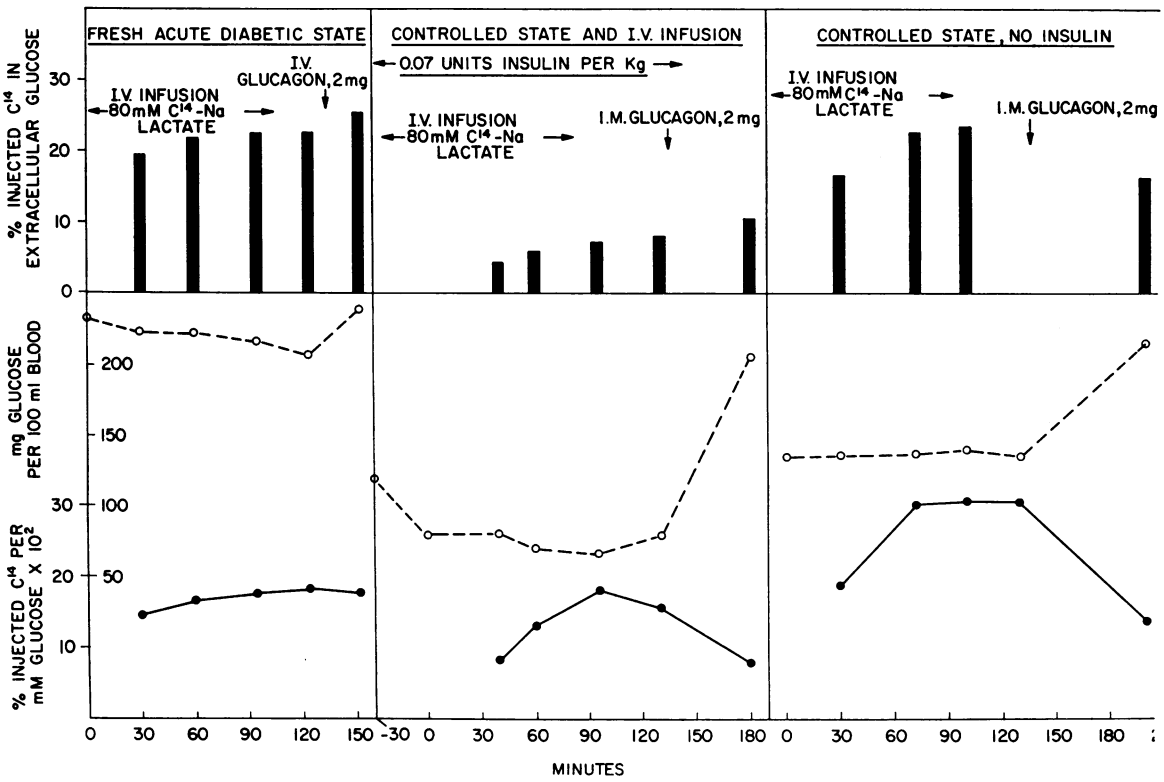

FIG. 2. GLUCOSE FORMATION FROM DL-LACTATE-2-C^{14} (LOAD) IN DIABETIC PATIENT R.P.

ure 2) than in any of the other patients (Figure 1, Table II). This may reflect a greater effect on hepatic glucose output of insulin given by slow intravenous infusion than by rapid injection, as observed by Madison, Combes, Adams, and Strickland (17). Hypoglycemia developing in the insulin study with R.P. may have caused glycogenolysis with resulting lowered C^{14} activity in glucose. The degree of elevation of blood glucose concentration after injection of glucagon and the fact that this glycogen was evidently labeled (increase in extracellular glucose-C^{14}) in the insulin study constitute some evidence against the occurrence of glycogenolysis before glucagon. Although the main hepatic action of glucagon is supposedly glycogenolysis, its gluconeogenetic effect (20) may further complicate interpretation in this study. Insulin's effect of lowering the extracellular glucose-C^{14} can nevertheless be demonstrated in diabetics (W.G. and J.D., Table II) under circumstances where the blood sugar falls, but no hypoglycemic levels are reached, and therefore presumably no adrenalin stimulation of glycogenolysis should occur.

Excessive gluconeogenesis in study I in R.P. could be related to the ketoacidotic state, which may be accompanied by excess glucocorticoids (21, 22), known to promote gluconeogenesis from pyruvate (22). Since in study III in R.P., without insulin infusion, but with relatively good diabetic control, almost as much glucose-C^{14} was formed as in study I, the primary cause for the reduction of extracellular glucose-C^{14} in study II in R.P. can be assumed to be the administration of insulin rather than the control of ketoacidosis with attendant decrease in circulating glucocorticoids.

When tolbutamide was employed in study III with the juvenile-type, insulin-dependent diabetic J.D., there was no lowering of blood sugar and also no decrease in extracellular glucose-C^{14} (Table II). This is further evidence that the effect of tolbutamide on hepatic glucose output is correlated with its over-all hypoglycemic action.

Table II shows that in a nondiabetic patient, M.B., less C^{14} appears in extracellular glucose than in untreated diabetics given lactate-2-C^{14}. The acute intravenous glucose load caused a moderate reduction in apparent C^{14} incorpora-

tion into glucose. Since glucose load distorts the sizes and relationships of glucose pools, comparison is more valid between the conditions of glucose load alone, in study II in M.B., and study III in M.B., in which both glucose load and tolbutamide preceded the C^{14} compound. Glucose disappeared faster after tolbutamide, and the incorporation of C^{14} into extracellular glucose was much less. Another difference between studies II and III in M.B. is seen in the labile glycogen labeling. C^{14} content of glucose after glucagon injection indicated plentiful glycogen synthesis in the glucose load study as compared with the control. After both glucose load and tolbutamide, however, glycogen appeared to be much less labeled than after glucose load alone.

Figure 3 shows that pyruvate-2-C^{14} is incorporated into extracellular glucose to the same extent and with similar differences among severe, mild, and nondiabetics as C^{14}-labeled lactate. The figure indicates low incorporation when a 25-g glucose load is infused during 30 minutes before isotope administration in a nondiabetic subject. The findings after glucagon administration again suggest relatively high labeling of glycogen compared with blood glucose after a glucose load.

DISCUSSION

It was shown earlier that severely diabetic patients incorporate twice as much C^{14} from acetate into extracellular glucose as nondiabetic or mild diabetic subjects (5). The present findings establish this observation with more direct and quantitatively important glucose precursors. They agree with other recent reports on incorporation of pyruvate-2-C^{14} into glucose of diabetic humans (6) and alloxan-diabetic rats (4), although the latter showed a more pronounced difference from normal. Other investigators have found increased conversion of C^{14}-palmitic acid to glucose in alloxan-diabetic rats (2) and decreased conversion of labeled serine or glycine to glucose after glucose load in normal rats (3). There are, however, reports of stimulated incorporation of $C^{14}O_2$ into glucose by insulin (23) and into glycogen by tolbutamide (24) that do not appear to agree with the present and other findings.

It seems significant that another type of measurement of hepatic glucose production *in vivo* in the undisturbed state, i.e., the rate of glucose turnover according to disappearance of blood glucose-C^{14} after single injection, has shown a rate two times higher than normal in severely diabetic humans (13, 14) and alloxan-diabetic rats (25).

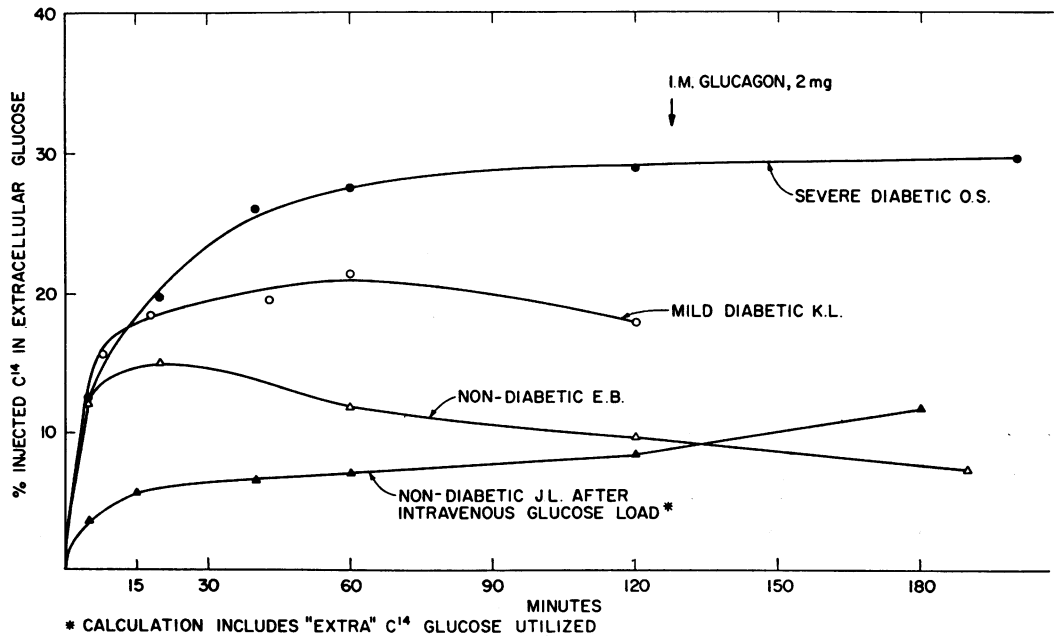

FIG. 3. APPEARANCE OF C^{14} IN BLOOD GLUCOSE AFTER IV INJECTION OF PYRUVATE-2-C^{14}

The magnitude of this change agrees with that calculated from the present studies.

Using blood vessel cannulation and glucose concentration measurement, some studies have failed to find decreased net hepatic glucose output after insulin (26–31), but not tolbutamide (30, 31). Others have noted a decreased output (17, 32), or even a net uptake (33) after insulin. Recent considerations (1) indicate that negative findings may relate to low carbohydrate in the antecedent diet, or to effects of surgery or anesthesia, as well as to the supervention of hypoglycemia and resulting glycogenolysis. Our patients all had carbohydrate intakes above the minimum required to sustain normal glucose tolerance in nondiabetic humans (34) and were studied under conditions of minimal stress. Hypoglycemia developed in some, but not all of our studies in which insulin appeared to decrease the hepatic output of glucose-C^{14}.

Accelerated uptake of circulating glucose by the liver, as an early effect of insulin or tolbutamide, could dilute newly formed C^{14}-glucose within the hepatic cell and thus lower the specific activity of that glucose being released by the liver. Recent demonstrations of increased uptake (33, 35, 36) therefore still leave in doubt the correct interpretation of our findings in regard to the actual effect of insulin or tolbutamide on gluconeogenesis.

Our studies show with considerable certainty that glucose load or insulin or tolbutamide do not completely arrest the production of new glucose by the liver, as was suggested by those workers (37, 38) who found the plateau effect on the rate of decline of glucose-C^{14} specific activity in the blood.

Most of the findings on the greater effect of tolbutamide than insulin on decreasing glucose-C^{14} formation could be explained as due to the stimulation by tolbutamide of secretion of endogenous insulin, which by direct transport to the liver via the portal vein has a more selective and pronounced action on the liver than on peripheral tissues. The one study, however, that superimposed tolbutamide on glucose load suggests that tolbutamide may have a more particular action to depress gluconeogenesis. Glucose load is followed by higher insulin levels in pancreatic venous blood than is tolbutamide (39), yet tolbutamide and glucose load clearly had a much greater action than glucose load alone to decrease C^{14} appearance in glucose. Also, in the glucose load study there was apparently the typical effect of insulin to promote glycogenesis, as indicated by release of C^{14}-labeled glucose by glucagon, but with tolbutamide acting concurrently, relatively little glycogen was deposited from newly formed glucose.

Aside from hormonal effects, the extent and rapidity with which circulating lactic and pyruvic acids are converted to circulating blood glucose impressively uphold the old physiological concept of the Cori cycle. Since the turnover time for blood glucose in humans is about 2 hours (13, 14), to the C^{14} calculated to be present in extracellular glucose at that time should be added a significant fraction formed and already metabolized, and an undeterminable amount deposited as glycogen. In severely diabetic subjects, therefore, possibly as much as 50% of circulating lactate and pyruvate is converted to glucose by the liver.

Since pyruvic acid-C^{14} showed the same range of differences among severe, mild, and nondiabetics in the incorporation into glucose, it seems that the mixed DL-form of lactic acid truly reflected the physiological and hormonal changes. The studies of Hoberman and D'Adamo (40) indicated that D-lactic acid is a carbohydrate precursor. Only 10% as much C^{14} from the 2 position of D-lactate was found in glycogen as from the correspondingly labeled L-lactate, the isomer found naturally in blood, although total glycogen yield from D-lactate appeared to be 40% as high. If D-lactic acid is converted to blood glucose more slowly or to a lesser extent than L-lactic acid, then the percentage of the natural form undergoing the transformation is even higher than we have estimated.

SUMMARY

When either DL-lactate-2-C^{14} or -3-C^{14} or pyruvate-2-C^{14} was injected intravenously, the appearance of C^{14} in blood glucose within the next 2 hours was in direct proportion to the existence and severity of the diabetic state, with about a twofold range between nondiabetic and severely diabetic patients. Nondiabetic subjects showed 10 to 15% of the injected C^{14} in calculated extracellular glucose and severe diabetics, up to 30%.

When insulin was given by rapid intravenous injection 10 minutes before labeled compound to

four diabetic patients, there was a decrease of appearance of C^{14} in extracellular glucose to approximately one-half the control amount. Insulin given by slow infusion to one diabetic patient, who also received the lactate-C^{14} in an intravenous lactate load, had a more pronounced effect.

Tolbutamide given intravenously 15 to 20 minutes before DL-lactate-3-C^{14} in two mild diabetic patients had about the same effect as insulin on the amount of C^{14} appearing in glucose, but caused much slower fall in blood glucose level. A 25-g intravenous glucose load in two nondiabetic patients had an effect similar to that of insulin or tolbutamide in diabetic patients. The prior injection of both tolbutamide and glucose load to one of these patients, however, resulted in much less C^{14} in glucose (from DL-lactate-2-C^{14}) than after glucose load alone.

Comparison of blood glucose levels and C^{14} content before and after glucagon administration suggested that insulin and glucose load promoted hepatic glycogen formation, but that tolbutamide did not.

Changes after insulin, tolbutamide, or glucose load could relate either to depressed gluconeogenesis, increased hepatic glucose influx, increased size of glucose space, or (in some insulin studies with hypoglycemia) to hepatic glycogenolysis. Differences between tolbutamide and the other agents suggested a more particular effect of tolbutamide on gluconeogenesis.

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