JCI The Journal of Clinical Investigation

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Edward W. Moore, … , Marvin L. Mitchell, Thomas C. Chalmers

J Clin Invest. 1959;38(7):1222-1227. https://doi.org/10.1172/JCI103897.

Research Article

VARIABILITY IN ABSORPTION OF INSULIN-I^{131} IN NORMAL AND DIABETIC SUBJECTS AFTER SUBCUTANEOUS AND INTRAMUSCULAR INJECTION *

By EDWARD W. MOORE,† MARVIN L. MITCHELL AND THOMAS C. CHALMERS

(From the Medical Services and Radioisotope Unit, Lemuel Shattuck Hospital and The Harvard and Tufts University Medical Schools, Boston, Mass.)

(Submitted for publication January 16, 1959; accepted March 27, 1959)

Variability in plasma binding and in peripheral utilization of insulin has been demonstrated in insulin-treated subjects (1, 2). Rates of insulin absorption in diabetics have previously been estimated by noting the insulin effect on the concentration of sugar in the blood, but absorption after subcutaneous and intramuscular injection has not been directly studied in the past. Its importance lies in the possibility that variation in absorption rate attributable to tissue binding or local vascular changes might be one of the factors responsible for alterations in the apparent sensitivity of diabetic patients to insulin.

The present study was undertaken to determine the rate of absorption and its variability in diabetics and in normals and to ascertain whether absorption rates are in any way dependent upon the site of injection. This was accomplished by measuring the disappearance time of insulin-I^{131} from subcutaneous and intramuscular injection sites in 22 diabetic and 11 normal subjects.

MATERIALS AND METHODS

Subjects consisted of hospitalized diabetic patients and volunteer hospital personnel who were apparently in good health. Crystalline beef insulin-I^{131} was obtained from Abbott Laboratories. The lots employed contained an average of one iodine atom per molecule of insulin (molecular weight 6,000). Injections of 0.05 to 0.4 unit of insulin containing 15 to 30 μc. insulin-I^{131} were administered subcutaneously or intramuscularly a total of 92 times to 11 normal and 22 diabetic subjects. The volume of the injected solution ranged from 0.02 to 1.0 ml., adjusted with phosphate buffer (pH 7.39) or, in some instances, with buffer plus nonradioactive (carrier) insulin.

The ages of the diabetic patients ranged from 25 to 70 years and their daily insulin requirements varied from 15 to 70 units. One patient (J.T.) was considered to have "brittle" diabetes and in another (J.M.) the presence of excessive circulating insulin antibodies had been demonstrated one year previously (3). None of the patients showed clinical evidence of lipodystrophy and their diabetes was well controlled during the time of study.

Each experiment was begun at 9 a.m., one and one-half to two hours after daily therapeutic insulin had been given and approximately one hour following breakfast.

Injections were administered in a standardized fashion into the deltoid area of each subject. Usually, simultaneous injections were given in each arm. External monitoring over the injection site was performed immediately and thereafter at hourly intervals for eight hours and in most instances at the end of 24 hours, using a collimated scintillation detector at a distance of 33 cm. With this technique, thyroid radioactivity was excluded and duplicate one minute counts had an average variability of 1.1 ± 1.0 per cent (1 S.D.). Radioactivity at the injection site was then expressed as percentage of initial count. For comparison, the time for absorption of half the radioactivity was used, hereafter designated as $T_{1/2}$.

The *in vitro* stability of the I^{131}-insulin linkage has been demonstrated previously (4, 5, 6). It was assumed that labeling was homogeneous and that disappearance of radioactivity paralleled absorption of intact insulin-I^{131}.

For comparative purposes, NaI^{131} was administered to four of the diabetic subjects and albumin-I^{131} to three additional normal subjects.

RESULTS

Insulin-I^{131} was injected subcutaneously 15 times into 11 normal subjects using two different volumes for injection. One group received a small volume (0.08 to 0.09 ml.), while the other was given an equivalent insulin dose diluted to 0.45 ml. with phosphate buffer. As shown in Figure 1, absorption was approximately exponential in both groups and tended to be more rapid with the larger volume. The mean $T_{1/2}$ for the small-volume group was 213 ± 16 minutes [1]

* This study was aided in part by a grant from the Schering Corporation, Bloomfield, N. J.

† Present address: National Cancer Institute, National Institutes of Health, Bethesda, Md.

[1] This and all following ± symbols refer to one standard error of the mean.

TABLE I

Variability in insulin-I^{131} absorption in six diabetic patients studied on more than one occasion

Patient	Date	Volume injected		Insulin-I^{131} per injection	Subcutaneous $T_{\frac{1}{2}}$		Intramuscular $T_{\frac{1}{2}}$	
		Right arm	Left arm		Right arm	Left arm	Right arm	Left arm
		ml.	*ml.*	*μc.*	*min.*	*min.*	*min.*	*min.*
A. C.	Jan. 14	0.15	0.15	22	261	348		
	Jan. 25	0.03	0.03	23	160			108
	Feb. 11	0.45	0.45	15		127	137	
	Feb. 14	1.00	0.30	17	110	110		
F. S.	Jan. 25		0.28	22		305		
	Feb. 14	0.80	0.20	17	98	94		
	June 26	0.51		10	181			
G. C.	Jan. 28	0.05	0.05	29		23½ hrs.	155	
	Jan. 31	0.02		9	211			
G. S.	Jan. 28	0.05	0.05	28	186	144		
	Feb. 11	0.45	0.45	15		126	118	
J. T.	Jan. 15	0.05	0.05	19	88	123		
	Jan. 30	0.03	0.03	23		84	140	
	Feb. 12	0.45	0.45	17		78	95	
	Feb. 15		0.45	17		83		
	May 31	0.45	0.45	12	53	71		
E. D.	Dec. 10	0.03	0.03	20	160	16 hrs.		
	Jan. 25	0.03	0.03	23	190			145
	Feb. 15	0.45	0.45	17		16½ hrs.	54	

and was significantly greater than the mean of 130 ± 16 minutes in the large-volume group ($p < 0.01$).

Twenty-two diabetic patients were similarly given a total of 50 injections, using volume schedules similar to those employed in the normal group (Figure 1). A total volume of 0.02 to 0.30 ml. was injected subcutaneously 18 times into 10 diabetics and volumes of 0.45 to 1.0 ml., containing comparable μc. concentrations, were administered 32 times to 19 diabetics. As in the normal subject, disappearance of radioactivity tended to be more rapid in diabetics when the larger volumes were employed. The mean $T_{1/2}$ of 173 ± 18 minutes in the small-volume group was not significantly greater than the mean of 146 ± 12 minutes in the large-volume group, however ($p > 0.1$). Individual variation in absorption rate was greater in diabetics, with a range in $T_{1/2}$ of 53 to 348 minutes as compared to 90 to 282 minutes in the normal group. Absorption in a given patient appeared to vary considerably on different occasions and to vary from arm to arm with simultaneous injection (Table I).

Markedly delayed absorption was demonstrated in two diabetic patients, omitted from the above means. In one of these the $T_{1/2}$ was approximately 23.5 hours, while in the other it was slightly over 16 hours on two occasions. In each instance, however, a normal rate of disappearance was observed simultaneously in the contralateral arm (Table I). The patient with "brittle" diabetes had consistent but rapid absorption rates (Table I). The patient with previously demonstrated insulin resistance (J.M.) had a modal absorption half-time.

Using comparable volumes for injection and omitting the two patients with markedly prolonged absorption, no significant difference could be demonstrated between normals and diabetics. The mean $T_{1/2}$ in the normal, regardless of the volume injected, was 160 ± 17 minutes as compared to 156 ± 10 minutes in the diabetic. No correlations could be found between absorption rate and insulin requirement, body weight, duration of the disease or specific activity (age) of the insulin employed.

In an effort to investigate possible sources of the observed variability, individual variations in subcutaneous absorption rate were studied by the

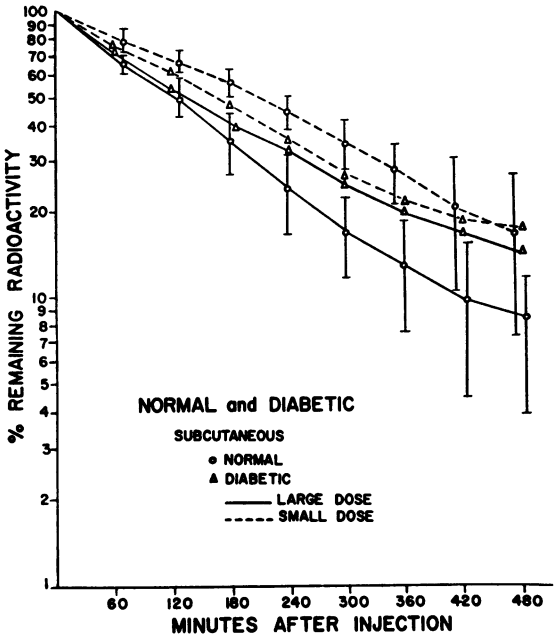

FIG. 1. COMPARISON OF ABSORPTION FROM SUBCUTANEOUS SITES IN NORMALS AND DIABETICS ACCORDING TO VOLUME INJECTED

Means ± 2 S. E. for normal groups are represented by circles and means for diabetic groups by triangles. Large volumes (0.45 to 1.0 ml.) are represented by solid lines; small volumes (0.02 to 0.30 ml.) by broken lines. Absorption half-times of more than 16 hours, found on three occasions in two patients, are omitted from these and subsequent curves and averages.

simultaneous administration of equal insulin doses into both arms in four normal and 12 diabetic subjects. In the normal group, the mean absolute difference in absorption half-times between arms for the simultaneous injections was 44 minutes (range of differences, 40 to 47 minutes). In the diabetic group it was 34 minutes (range of differences, five to 87 minutes). Although the range of differences between arms was greater in the diabetics, the average of these differences did not vary significantly from that of the normal group.

Simultaneous intramuscular and subcutaneous injections were given to seven normal and five diabetic subjects. Results are shown in Figure 2. In both normals and diabetics, absorption half-times were somewhat less after intramuscular injection and there was also less individual variation by this route. The mean $T_{1/2}$ in the normal group was 130 ± 17 minutes subcutaneously and 88 ± 7 minutes intramuscularly, as compared to 142 ± 19 minutes and 123 ± 6 minutes, respectively, in the diabetic group. No significant difference was noted between subcutaneous and intramuscular absorption half-times in either group ($p > 0.05$). The mean intramuscular $T_{1/2}$ of 123 minutes in the diabetic group was significantly greater than the mean of 88 minutes in the normal, however ($p < 0.01$). "Tailing" of the intramuscular curves after four hours was noted in both groups.

In eight diabetics, a subcutaneously administered tracer dose diluted with phosphate buffer was compared with a simultaneous injection in which 10 to 20 units of nonradioactive (carrier) insulin with added phosphate buffer was used as diluent (Figure 3). Although the addition of carrier prolonged the mean $T_{1/2}$ from 191 ± 26 minutes to 240 ± 22 minutes (mean difference, 49 ± 18 minutes, $p < 0.05$), this was largely due to delayed absorption in the first two hours and the configuration of the absorption curves was similar thereafter.

The amount of radioactivity remaining at the in-

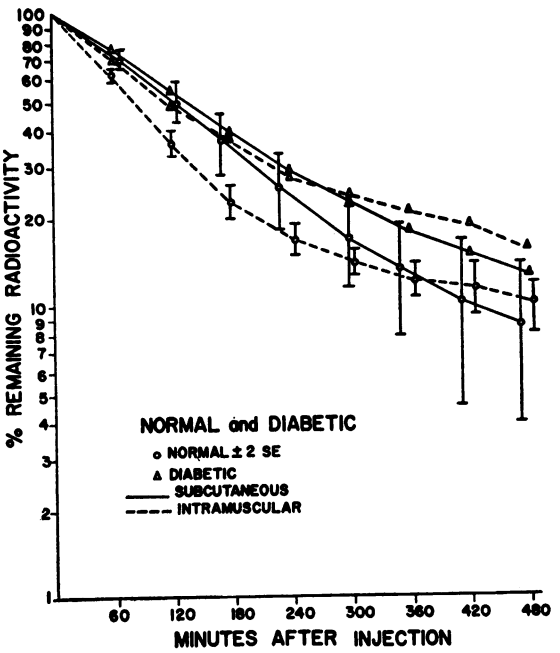

FIG. 2. COMPARISON OF SUBCUTANEOUS AND INTRAMUSCULAR ABSORPTION IN NORMALS AND DIABETICS

Means ± 2 S. E. for normal groups are represented by circles; means for diabetic groups by triangles. Subcutaneous routes are represented by solid lines; intramuscular routes by broken lines.

jection site after 24 hours was determined in five normal and six diabetic subjects. In the normal group, a mean of 1.5 ± 0.4 per cent of initial radioactivity remained following subcutaneous injection. This was significantly less than the mean of 4.1 ± 0.9 per cent noted after intramuscular injection ($p < 0.01$). In the diabetic group, means of 2.5 ± 0.3 per cent and 8.2 ± 1.8 per cent remained at the respective subcutaneous and intramuscular sites after 24 hours. This difference was also significant ($p < 0.02$). The amount of radioactivity remaining at subcutaneous sites was not significantly different in normals and diabetics ($p > 0.05$), but the mean of 4.1 per cent noted at intramuscular sites in the normal was significantly less than the mean of 8.2 per cent noted in the diabetic group ($p < 0.05$).

For comparative purposes, four diabetic patients were given simultaneous subcutaneous and intramuscular injections of NaI^{131} (Figure 4). Absorption was rapid and virtually complete within one hour. It was slightly faster intramuscularly (mean $T_{1/2} = 20$ minutes) than subcutaneously (mean $T_{1/2} = 25$ minutes), but the difference was not significant.

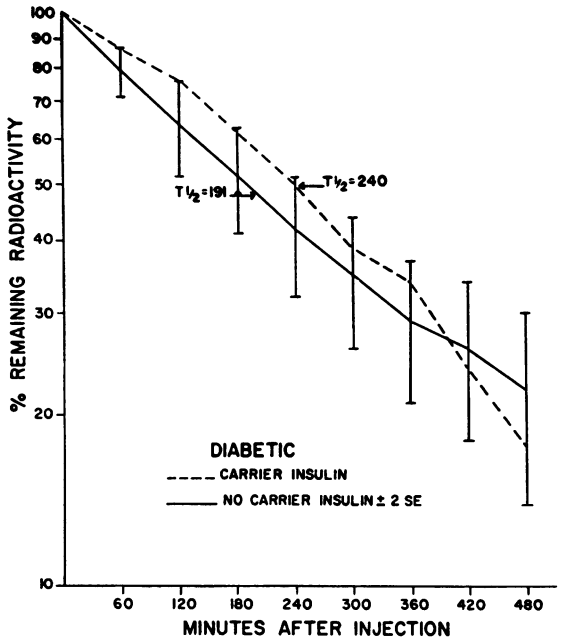

FIG. 3. EFFECT OF DILUTION OF SUBCUTANEOUSLY ADMINISTERED TRACER INSULIN-I^{131} WITH NONRADIOACTIVE CARRIER INSULIN

Mean ± 2 S. E. without carrier is represented by solid line; with added carrier, by broken line.

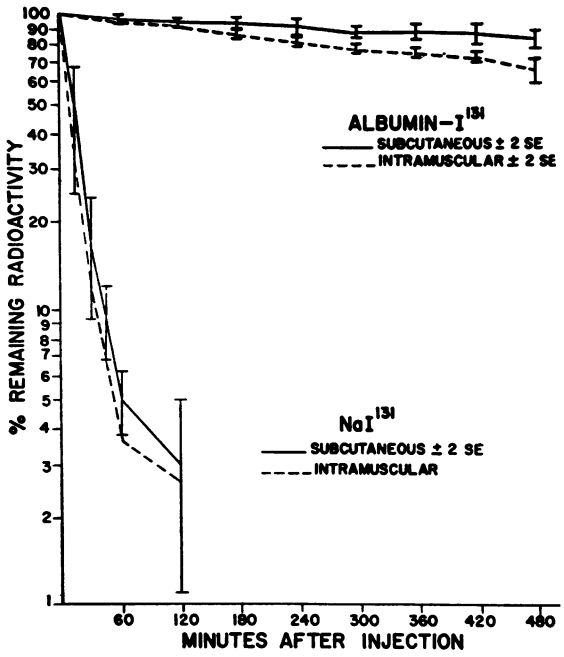

FIG. 4. SUBCUTANEOUS AND INTRAMUSCULAR ABSORPTION OF ALBUMIN-I^{131} IN THREE NORMAL SUBJECTS AND OF NaI^{131} IN FOUR DIABETICS

Means ± 2 S. E. for subcutaneous routes are represented by solid lines; intramuscular routes by broken lines.

Albumin-I^{131} was administered subcutaneously and intramuscularly to three normal subjects (Figure 4). Absorption was considerably slower than it was with insulin-I^{131}. After two hours, disappearance of radioactivity from the intramuscular site was significantly faster than it was from the subcutaneous site ($p < 0.01$), and by extrapolation, $T_{1/2}$ was estimated to be approximately 13 and 22 hours by the respective routes.

DISCUSSION

The presence of insulin-binding moieties in the circulating plasma proteins of insulin-treated subjects is well documented (1–3, 7–14). These moieties have been considered to be antibodies (2, 11–13) and usually have been found to be associated with the gamma and beta globulin fractions of the plasma proteins (2, 3, 9, 10–13). A correlation between insulin requirement and serum in-

sulin-inhibitory properties has been shown in some instances (3, 9, 11).

In the present study, disappearance of radioactivity from injection sites has been equated with insulin absorption. As noted by Scott, Prout, Weaver and Asper (15), a study of insulin metabolism with insulin-I^{131} is based on the assumption that the radioactive insulin is homogeneously labeled, is unaltered in the labeling process and that it is metabolized in the same manner as endogenous insulin. Berson and co-workers (2) have found that a variable portion of insulin is physically altered in the labeling process. As pointed out by Haugaard, Vaughan, Haugaard and Stadie (5), radioactivity in tissues is a measure of their radioisotope content and is not necessarily a true measure of the isotopic insulin present. Any breakdown products of insulin containing radioactive label, as well as free radioactive iodine, will be measured in addition to molecules of the originally injected insulin. For comparative purposes, however, these factors would not appear to be of major consequence. The persistence of significant amounts of free I^{131} at the site of injection seems unlikely in view of the rapid absorption demonstrated with NaI^{131}.

If local breakdown of iodinated insulin occurs and is enzyme-dependent (*i.e.*, not random), one would expect a retardation in disappearance of the label once the maximum capabilities of the enzyme system were exceeded. It was shown, however, that dilution of labeled insulin with phosphate buffer tended to hasten absorption in both normals and diabetics, perhaps related to the higher pH in the buffered system or to an easier access to the bloodstream. Addition of nonradioactive carrier insulin somewhat prolonged the disappearance time of the label.

In the present study, no significant difference between subcutaneous and intramuscular absorption half-times could be demonstrated in either normals or diabetics. When diabetics were compared with normals, subcutaneous absorption rates were found to be similar, although intramuscular absorption was significantly faster in the normal group.

Berson and co-workers (2) have suggested that fluctuations in the production of "insulin transporting" antibodies may be causally related to the lability of insulin requirements in those patients who manifest what has been termed "brittle" diabetes. It is also possible that these or other antibodies may be present locally at the site of insulin injection, either cell-bound or free in the extracellular fluid. Since the above authors (2) found maximum plasma binding in nonresistant patients to be in the range of only a few units per L. of plasma, the total amount of antibody at an injection site would probably be small in relation to the doses of insulin employed therapeutically or in the present study (0.05 to 0.4 unit). The similar absorption half-times in normals and diabetics following subcutaneous injections would indicate that local insulin antibodies, if present, are probably of no therapeutic significance.

The fact that the mean intramuscular $T_{1/2}$ in diabetics was significantly greater than in the normals, coupled with the finding of significantly greater radioactivity after 24 hours, could be interpreted as evidence for "tissue binding." The tailing of the intramuscular curves also indicates some binding at that site which is not present in the subcutaneous tissues. Since this was noted in both normals and diabetics, it could hardly be attributed to prior insulin therapy and would not be likely to represent insulin-specific antibodies. It cannot be stated at the present time whether or not this radioactivity represents undegraded insulin.

Considerable variability in the rate of insulin absorption from subcutaneous tissues has been demonstrated. Markedly prolonged absorption was noted in two diabetic patients, and in one of these it occurred on two different occasions. In each instance, however, a normal absorption pattern was simultaneously observed in the contralateral arm, suggesting that local factors were responsible. No hematoma or other abnormality was grossly evident in these cases and it was assumed that the injections had been given into relatively avascular tissues. Whatever the mechanism, it seems reasonable to assume that such occurrences in the therapeutic administration of insulin might account, in part at least, for fluctuations in the control of some diabetic patients.

SUMMARY AND CONCLUSIONS

1. Insulin-I^{131} was administered subcutaneously or intramuscularly to 11 normal and 22 diabetic subjects and rates of disappearance of radioactivity from the injection sites were determined.

2. Absorption was approximately exponential but there was marked variation in rates of disappearance from subcutaneous tissues. Less variation was noted following intramuscular injection.

3. No significant difference between subcutaneous and intramuscular absorption half-times was found in either normals or diabetics.

4. Intramuscular absorption was significantly faster in normals than in diabetics and at 24 hours the residual radioactivity at intramuscular sites was significantly less in the normals.

5. "Tailing" of intramuscular curves was noted in both normals and diabetics and suggested some tissue binding in that site which was not present in the subcutaneous tissues.

6. Dilution of the administered tracer dose with phosphate buffer shortened mean half-times. The addition of carrier prolonged absorption somewhat, but results were inconclusive.

7. Extremely prolonged absorption was demonstrated in two diabetic patients, in each of whom a normal disappearance rate was simultaneously observed in the contralateral arm, suggesting that local factors, perhaps relative avascularity, were responsible. Such occurrences in the therapeutic situation may account in part for fluctuations in the control of some diabetic patients.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. Joanne Earley for her technical assistance and Dr. Edmund A. Gehan of the Experimental Statistics Section of the National Cancer Institute for his assistance in the statistical analysis.

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