Glucose Kinetics During Oral Glucose Tolerance Test in Normal, Methylprednisolone-Treated and Alloxan Diabetic Dogs

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SUMMARY

Using 2-^{3}H-glucose as tracer, glucose turnover was measured (primed constant rate infusion) on dogs with indwelling arterial and venous catheters. Normal, methylprednisolone(MP)-treated (three days, 3 to 3.2 mg./kg.) and alloxan diabetic dogs were given 2 gm./kg. glucose (in 200 ml. water) containing 2.3 μCi./gm. of ^{14}C-glucose (U) to drink. The rate of absorption of glucose was calculated from the course of the specific activity of ^{14}C-glucose in the plasma. In normal and MP-treated dogs, the cumulative absorption of glucose could be described as a linear function of time for t < 120 minutes. The glucose absorption in alloxanized dogs showed an early peak with a subsequent decline. In all three types of dogs a single exponential function described the glucose absorption for t > 90 minutes with an asymptotic value of about 2 gm./kg. In normal and glucocorticoid-treated dogs, the glucose load decreased the hepatic glucose output by 50 and 70 per cent, respectively. The metabolic clearance rate (MCR) of glucose was greatly increased in both groups. There was a log-to-log correlation between MCR and immunoreactive insulin in the plasma. In alloxan diabetes the low glucose tolerance was due to two factors: (a) the glucose drink failed to suppress the hepatic glucose output, and (b) it increased the clearance rate of glucose (the sum of MCR and renal clearance) very little. DIABETES *23*: 645-50, August, 1974.

The most commonly used oral glucose tolerance test (OGTT) consists of drinking 1.75 gm./kg. glucose and the evaluation is based on the blood glucose concentration measured two hours later.[1] From the point of view of glucose kinetics the outcome of the test depends on (a) the rate and pattern of absorption of glucose from the intestine, (b) changes in the rate of hepatic glucose output (the sum of a + b represents the rate of appearance of glucose in the plasma), and (c) the rate at which glucose disappears from the plasma due to uptake by the tissues (including the liver) and renal loss of glucose if the plasma glucose rises higher than the renal threshold. The present paper is an attempt to quantitate each of these three factors by using two radioglucose tracers: (1) [glucose-^{14}C (U)] to measure the rate of absorption of the glucose, and (2) (glucose-2-^{3}H) to estimate the effect of oral glucose on the over-all glucose turnover. The study was carried out on normal, methylprednisolone(MP)-treated, and alloxan diabetic dogs. The latter two groups are characterized by highly elevated basal turnover rates of glucose. In the MP-treated dogs the high glucose turnover[2,3] is accompanied by a significantly elevated plasma insulin level with only a slightly increased blood sugar; in alloxan dogs the lack of insulin (due to destruction of β cells) is responsible for the increased glucose turnover in the presence of a pronounced hyperglycemia and glycosuria.

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METHODS

The study was conducted on mongrel dogs (body weight, 12 to 16 kg.). Three or four days before the experiment, under light morphine-Pentothal anesthesia, indwelling polyethylene catheters were placed in the left jugular vein and common carotid artery. All animals were fed Purina Dog Chow with 250 gm. meat added to it. Drinking water and feed were withdrawn twenty-four and eighteen hours, respectively, before the experiment. After a priming dose of 3 to 4 μCi./kg., 2-^{3}H-glucose was infused intravenously at a constant rate (30 to 40 nCi./kg. min.) for 300 minutes. Arterial blood samples were collected in chilled

tubes at 60, 75, 90 and 120 minutes after the priming dose to establish baseline values. After the two hour equilibrium period the animal was offered 200 ml. water containing 2 gm./kg. glucose and 2.3 μCi./gm. glucose-^{14}C (U). The experiment was continued only if the dog drank the glucose solution as well as a small amount of rinsing water within two to three minutes ($t = 0$). Following this, blood samples were taken at $t = 5$, 10, 15, then at fifteen minute intervals until $t = 90$, and finally at $t = 120$, 150 and 180 minutes.

The plasma proteins were precipitated with $Ba(OH)_2$ and $ZnSO_4$ and after centrifugation the glucose in the supernatant was separated from organic acids (mainly lactate and pyruvate) on columns of IRA-400 anion exchange resin[4] (Mallinckrodt Chemical Works, St. Louis, Mo.). Two milliliter aliquots of the eluate were evaporated to dryness in vacuum at 75° C. in order to eliminate the tritiated water present in the plasma. The residue was dissolved in 1 ml. water and counted in Aquasol (New England Nuclear) in the Packard Liquid Scintillation Spectrophotometer according to the double label counting technics. Internal standards were used to correct for quenching and "spill" of ^{14}C counts into the tritium channel. The glucose content in aliquots of the eluate was measured with the glucose oxidase method. All measurements were carried out in duplicate and the specific activities (SAH and SAC) were expressed in nCi./mg. glucose. Three animals served as controls and four dogs were treated with MP for three consecutive days (intramuscular Depo-Medrol 3 to 3.2 mg./kg. per day). Four animals were given alloxan 70 to 80 mg./kg. intravenously. All diabetic dogs were maintained on Protamine Zinc insulin in daily doses as required to prevent glucosuria (1 to 1.5 U./kg. per day). The last dose of insulin was a short-acting preparation administered forty-eight hours before the experiment. In a number of experiments, plasma insulin level was measured according to the radioimmunoassay of Hales and Randle.[5]

Calculations

In steady state, when the glucose level (gm., mg./ml.) and the specific activity of glucose (SAH, nCi./mg.) are constant, the rate of appearance of glucose (Ra, mg./kg. min.) in the miscible pool can be calculated simply by dividing the infusion rate of the tracer (F, nCi./kg. min.) by SAH (Ra = F/SAH). Since the glucose level is constant, Ra = Rd (rate of disappearance of glucose). These conditions are reasonably met in the second hour of the baseline period.

In nonsteady state (after the oral glucose) Ra was calculated according to the general principles outlined by Steele[6]

$$R_a = \frac{F - Vg\,\frac{dSAH}{dt}}{SAH} \qquad \text{(Equation 1)}$$

where "V" (ml./kg.) is the volume of distribution of glucose. A previous study[7] carried out with the two tracer technic showed that in the early phase of a perturbation of glucose turnover (due to glucose or glucagon infusion) the value of "V" is less than the glucose space, and it rises, rapidly approaching an asymptotic value. Its course between $t = 5$ and $t = \infty$ could be described by the equation

$$V_t = 303\text{-}167.59e^{-0.045t}$$

If Ra is known, the rate of absorption of glucose (Rabs, mg./kg. min.) can be calculated because the rate at which ^{14}C-glucose enters the plasma can be expressed as the products: Rabs x SAD (=specific activity of the glucose drink). Therefore, substituting this for F in equation 1 and solving it for Rabs

$$R_{abs} = \frac{R_a \,.\, SAC + Vg \,.\, dSAC/dt}{SAD} \times f$$

"f" is a conversion factor made necessary by the fact that Ra measured by 2-^{3}H-glucose is higher than that obtained with glucose-^{14}C(U);[8] ^{14}C-glucose, unlike 2-^{3}H-glucose, gives rise to radiolactate, radiopyruvate, etc. and is incorporated into liver glycogen.[9] Therefore, the radiocarbon recycles partly via gluconeogenesis from C_3 units (lactate, pyruvate, alanine) and partly from the outer tiers of liver glycogen.[10] If no priming precedes the infusion of ^{14}C-glucose (no equilibrium exists), then there is a linear and inverse correlation between SAC and "f"[7] probably due to the increasing recycling as SAC rises, and $f = 1.04\text{-}b \times SAC$.

In experiments with a simultaneous and equal rate infusion of 2-^{3}H-glucose, ^{14}C-glucose together with nonradioactive glucose (10 mg./kg. min.) yielded values of "b" in control dogs, MP-treated and alloxan diabetic animals of 0.06, 0.08 and 0.126, respectively.

Ra and Rabs can be calculated for any given time if a least square regression analysis provides the equations which adequately describe the course of the specific activity curves (SAH and SAC). This procedure has

the additional advantage that it does not consider the measured values of SA − s as errorless. The rate of disappearance of glucose (Rd, mg./kg. min.) was calculated for the period t_{45} to t_{165} when the arterial glucose level could be assumed to represent the average glucose concentration of the miscible pool. Then

$$Rd = Ra - V\frac{dg}{dt} \qquad \text{(Equation 3)}$$

Finally, the clearance rate of glucose (CR, ml./kg. min.) was calculated: CR=Rd/g. This represents the volume "cleared" of glucose per unit of time by "unidirectional pathways of metabolism, storage, or excretion".[11] If g <2 mg./ml. (renal threshold), CR is referred to as metabolic clearance rate (MCR) and it is a useful index of the ability of the tissues to remove glucose from the pool.

RESULTS

Table 1 summarizes the mean baseline values characteristic for the three groups of dogs. Both alloxan and MP treatment increased the glucose turnover. The CR, however, was much less in diabetic than in MP-treated animals even though in the former group CR=MCR + RCR (renal clearance rate) while in the MP group CR=MCR only.

Figures 1 and 2 show the effect of an oral glucose load on glucose kinetics in the same dog before (continuous lines) and six days after 70 mg./kg. alloxan (broken lines). Figure 1 presents the measured values (g, SA), the evaluation of which is shown in figure 2. The diabetic state is characterized by (a) the well known hyperglycemia, (b) the markedly elevated glucose turnover (in this case 3.7 versus 7 mg./kg. min.),

TABLE 1

Baseline values

	glucose mg./100 ml.	Ra mg./kg. min.	CR ml./kg. min.	IRI μU./ml.
Controls	103	3.8	3.7	12.1
± S.E.M. (n)	2 (12)	0.2 (8)	0.3 (8)	1.4 (10)
Alloxan	376	10.2	2.7	—
± S.E.M. (n)	57 (8)	1.5 (8)	0.2 (8)	—
MP	119	7.4	6.1	29.7
± S.E.M. (n)	3 (12)	0.5 (7)	0.3 (7)	4.5 (8)
P <				
C → A	.001	.001	.02	—
C → MP	.001	.001	.001	.001
A → MP	.001	N.S.	.001	—

C = Controls; A = Alloxan; MP = Methylprednisolone

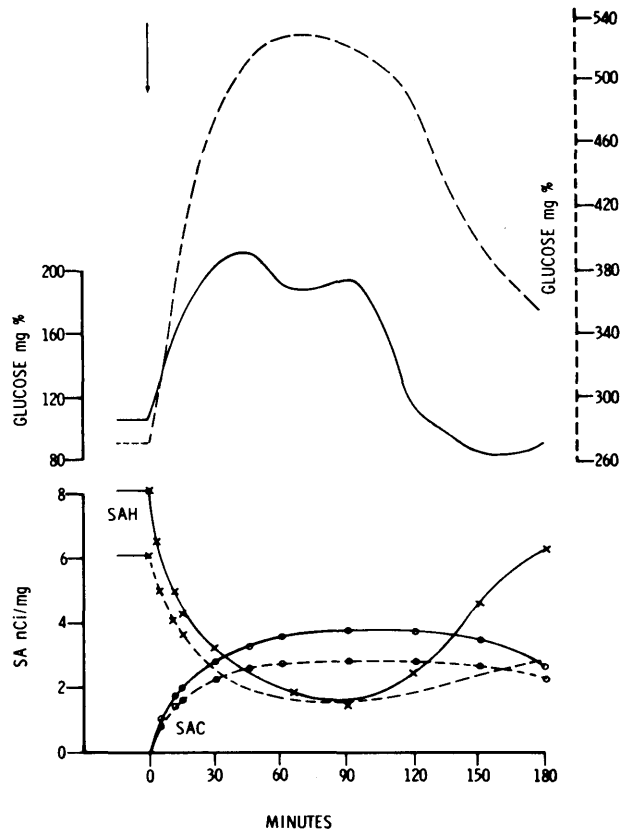

FIG. 1. Effect of oral glucose on glucose kinetics in the same dog before (—) and after 70 mg./kg. intravenous alloxan (----). Glucose turnover was measured with 2-^{3}H-glucose (primed constant rate infusion). After a baseline period of two hours at t = 0, 2 gm./kg. glucose with 2.3 μCi. ^{14}C-glucose (U)/gm. was given in 200 ml. water (arrow). SAH, SAC are the specific activities of 2-^{3}H-glucose and ^{14}C-glucose in the plasma, respectively.

and (c) the considerably lower than normal CR (in this case 2.2 versus 3.8 ml./kg. min.). OGTT showed the typical diabetic course of plasma glucose with a larger increment and delayed return (figure 1). In the first thirty to forty minutes the rate of absorption of glucose (or rather the rate at which the absorbed glucose appeared in the arterial plasma) was more rapid after alloxan than under control conditions (figure 2). Later, however, Rabs falls below the control level. Calculation of Rabs and Rd showed that the delayed, diabetic, return of plasma glucose cannot be explained either by a more rapid absorption or by a lower than normal rate of disappearance of glucose. In fact, in the third hour Rd was considerably higher in the diabetic state than prior to alloxan. The clearance rate (CR) and the rate of hepatic glucose output (RH) seem to be the two decisive factors which determine the tolerance

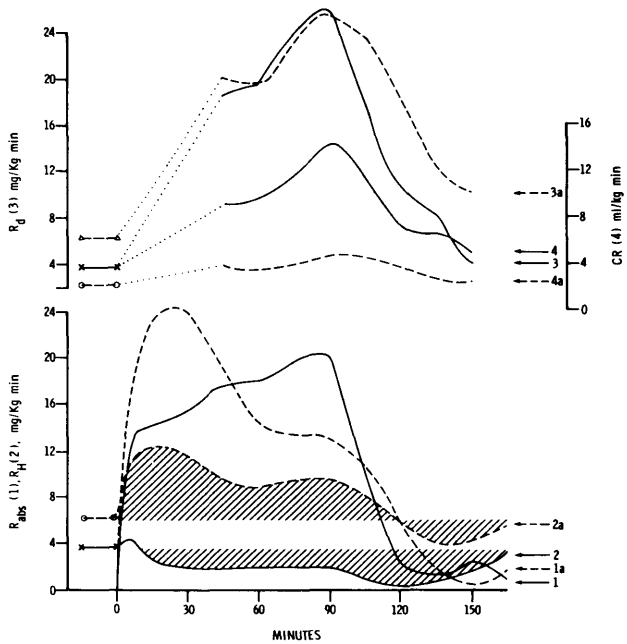

FIG. 2. Evaluation of the two experiments shown in figure 1: continuous lines (1-4), control; and broken lines (1a-4a), diabetic state. Lower panel: rates of absorption of glucose (R_{abs}), lines 1 and 1a; and hepatic glucose output (R_H); lines 2 and 2a. Shaded areas are deviations from the baseline values. Upper panel: disappearance rate of glucose (Rd), lines 3 and 3a; and clearance rates (CR), lines 4 and 4a. Rd and CR were not calculated for the period t_0-t_{45} (see text).

curve. Under normal conditions CR increased to some 10 to 11 ml./kg. min. and the hepatic output decreased markedly. After alloxan the CR increased but little, to about 4 ml./kg. min., and the oral glucose load failed to decrease the glucose output of the liver but rather increased it, in this case by more than 100 per cent. The average response of plasma glucose (Δ glucose) and CR in each of the three groups of experiments (controls, MP-treated and alloxan diabetic dogs) is shown in figure 3.

In alloxan dogs the drinking of 2 gm./kg. glucose caused a markedly greater increase of plasma glucose and a smaller rise of CR than in the other two groups. The difference is significant at the $p < .01$ level.

The effect of OGTT on the average response of hepatic glucose output and the course of glucose absorption is shown in figure 4. The former was markedly depressed in both the controls and MP-treated animals, but not in the alloxan diabetic dogs. The greatest contrast was found between the MP-treated and the diabetic group. Both of these groups had highly elevated baseline values of hepatic glucose output which was depressed by more than 70 per cent in the MP-treated animals and not at all in the diabetic ones. On the contrary, this latter group showed a tendency to increase the endogenous glucose output during OGTT.

The average course of glucose absorption was similar in the controls and MP-treated animals; the alloxan dogs, however, showed an earlier peak with a subsequent decline of Rabs in the second hour. The variation in Rabs with time made the difference between the groups statistically not significant. However, regression analysis of the cumulative absorption curve (table 2) revealed that (a) in the first forty-five minutes the average Rabs (slope constant "b" of the linear equation) in the alloxan dogs was significantly ($p < .001$) higher and, in the t_{45} to t_{120} minute period, was lower than in the two other groups; (b) the asymptotic value calculated with the Taylor[12] series was found to be essentially 2 gm./kg.; (c) the time required to reach

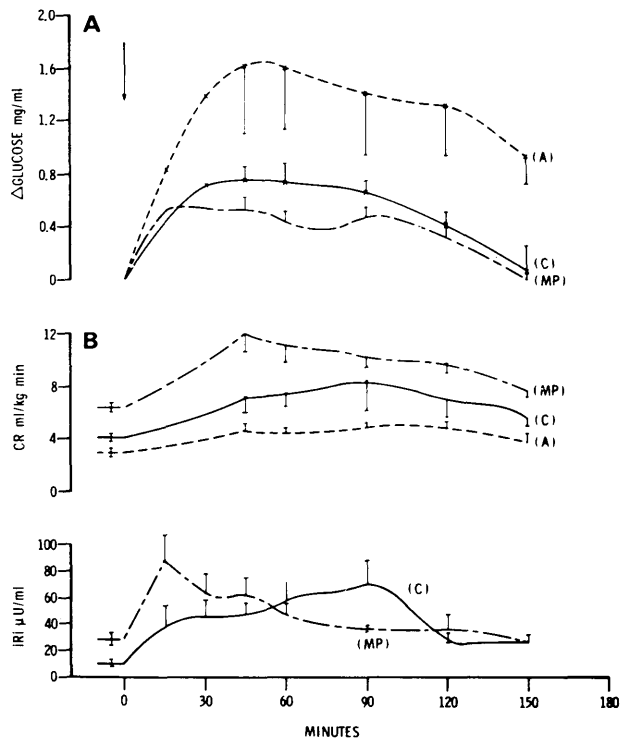

FIG. 3. A. Mean response of plasma glucose to oral glucose (2 gm./kg., at arrow) in controls (C:—, n = 9), methylprednisolone-treated (MP:—·—, n= 8), and alloxan diabetic (A: ----, n = 4) dogs. B. Upper curves: clearance rate of glucose (C, n = 3; MP, n = 4; A, n = 4); vertical lines are S.E.M. Between t_{45}-t_{150} the differences between MP and A as well as C + MP and A are significant at $p < .01$ level (Student "t" test). Lower curves: iRi: immunoreactive insulin, control and MP-treated dogs. Vertical lines: S.E.M.

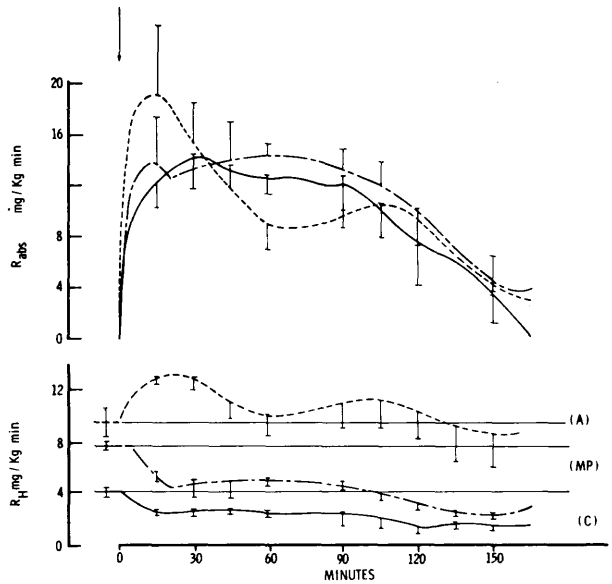

FIG. 4. *Mean course of the rate of absorption of glucose and of hepatic glucose output (RH). Symbols and number of experiments as in figure 3B.*

95 per cent of the asymptotic value in the controls, MP-treated and alloxan diabetic dogs was 236, 202 and 239 minutes, respectively. In other words, MP treatment increased the over-all absorption of glucose. The response of plasma insulin (IRI) to the OGTT in the controls and MP-treated dogs was not significantly different (figure 3). Statistical analysis revealed, however, a significant correlation ($r = 0.815$, $p < .001$) between the logarithm of MCR of glucose and the logarithm of plasma insulin level (figure 5). Values obtained from the MP-treated dogs were, on both sides of the regression line, within ± 1 S.D. of estimate.

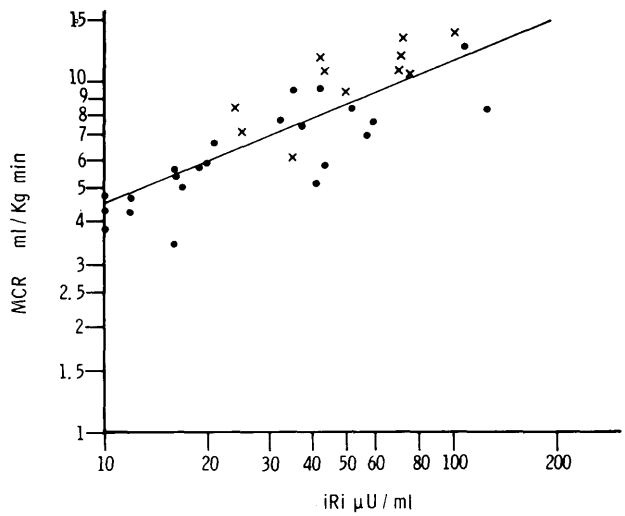

FIG. 5. **Log-to-log correlation between MCR and iRi in control (•) and MP-treated (x) dogs, MCR = 1.74 x $iRi^{0.41 \pm 0.05}$, r = 0.815; p < .001 (n = 36).**

TABLE 2

Regression analysis of cumulative absorption of glucose

for t < 120 minutes, y(mg./kg.) = a + b t

for t ≤ 45 min.

	a	b ± S.E.	S.D. of estimate (± mg./kg.)
Contr.	-57.4	13.1 ± 0.32	10
MP	-39.6	13.3 ± 0.08	3
Alloxan	-42.7	17.4 ± 0.21*	7

for 45 ≤ t ≤ 120 min.

	a	b ± S.E.	S.D. of estimate (± mg./kg.)
Contr.	28.1	11.8 ± 0.35	22
MP	-19.9	13.3 ± 0.31†	19
Alloxan	297.4	9.3 ± 0.26*	16

for t > 90 minutes y(mg./kg.) = a - be^{-ct}

	a	b	c ± S.E. x 10^{-4}	S.D.
Contr.	1,970	3,300	0.0149 ± 8.1	20
MP	2,023	4,580	0.0189 ± 4.1‡	10
Alloxan	1,993	3,260	0.0146 ± 2.6	10

*Relative to both control and MP $p < .001$
†Relative to control $p < .02$
‡Relative to control $p < .01$ and relative to alloxan $p < .001$

DISCUSSION

Our results on normal dogs essentially confirmed the observations of Steele et al.[13] who estimated the glucose turnover by using glucose-6-^{14}C and measured the absorption of 1 gm./kg. glucose containing glucose-^{14}C (U) administered by stomach tube. In both studies the oral glucose depressed the hepatic glucose output and increased the rate of disappearance of glucose, markedly increasing the metabolic clearance rate (MCR). Measurements of glucose kinetics during OGTT in alloxan dogs revealed two striking deviations from the normal response. First, the hepatic glucose output was not depressed; instead it showed a transient increase. The mechanism of this increase is not clear and it may be related to the findings that glucose infusion without insulin decreases liver glycogen in alloxan diabetic dogs.[14] Second, the clearance rate of glucose rose only slightly (probably due to an increase of renal loss of glucose caused by the large hyperglycemia). The decreased glucose tolerance in diabetes, namely, the greater increase and the de-

layed return of plasma glucose, can adequately be explained by these two factors. It should be noted that in diabetes, the rate of disappearance of glucose (without relating it to the glucose level) does not seem to provide any meaningful information because due to the higher glucose level, Rd is higher than normal (figure 2).

As to the effect of glucose load on the hepatic glucose output, one has to point out that 2-^{3}H-glucose does not measure net balance of glucose across the liver but rather the rate at which glucose leaves the liver. Consequently, the tracer technic will show glucose output even when the hepatic uptake is larger than the output[15] and the liver is accumulating glycogen. One may, however, interpret the earlier high peak of glucose absorption in alloxanized dogs as being caused by the absence of hepatic removal of the absorbing glucose. The subsequent decline of R_{abs} is probably due to the rapidly decreasing concentration gradient across the intestinal wall caused by the high plasma glucose (> 400 mg. per 100 ml.).

The overwhelming role of plasma insulin in OGTT was most strikingly demonstrated by the experiments on MP-treated dogs.

Despite the somewhat faster absorption, plasma glucose rose in MP-treated animals less than in the controls—which is in accordance with the observation that MP treatment increased the intravenous glucose tolerance in dogs.[16] The very high MCR and the strong suppression of hepatic glucose output seem to be responsible for this.

The moderate increase of glucose tolerance by MP is at variance with the marked reduction of OGT following a brief (two days) prednisolone treatment of normal human beings.[17] While no definite explanation can be offered, it seems likely that, apart from possible species differences, the hyperinsulinemia caused by the large doses of MP is responsible for this discrepancy.

Finally, it should be pointed out that the glucocorticoid treatment did not seem to interfere with the effect of insulin on the peripheral uptake of glucose. This conclusion is based on the observation that values from both controls and MP-treated animals fitted the same log-to-log correlation between MCR and IRI. The so-called "insulin resistance" due to glucocorticoids may well be the result of an elevated plasma glucagon level[18] and of a greatly increased sensitivity of these dogs toward the hepatic effect of glycogenolytic hormones, such as glucagon and epinephrine.[3,19] This would explain the highly elevated glucose turnover combined with an increased glucose tolerance.

ACKNOWLEDGMENT

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