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Hormone-Fuel Interrelationships during Fasting *

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Over 50 years ago, Benedict (2) published his extensive monograph on the metabolism of fasting in man, in which he demonstrated that carbohydrate stores provide a small but significant component of the body's fuel for only the first few days. Thereafter, protein and fat are the sole sources of fuel, the former contributing 15% of the calories and the latter the balance.

The primary role of fat as fuel was apparent to Benedict and his contemporaries; it is plentiful and expendable. The significance of the protein requirement, however, was less clear; in fact, it was not fully understood until nearly 20 years later when the obligatory dependence of the central nervous system on glucose was firmly established (3). Since glycogen stores in man were known to approximate only 200 g, it was readily apparent that glucose has to be derived from protein in order to maintain cerebral metabolism during a prolonged fast. More recently, our understanding of the fasted state has been further clarified by the demonstration that free fatty acid is both the major transport form of lipid leaving adipose tissue (4, 5) and a substrate that is readily utilized by liver, muscle, and many other tissues.

Although the above findings provide a basis for understanding the metabolism of fasting, certain areas such as the physiologic role of hormones and the mechanisms controlling glucose production and utilization remain poorly defined. In addition, estimates of glucose turnover (6–12) or splanchnic glucose production (13–15) during a short fast all greatly exceed the amount that can be contributed by gluconeogenesis (as reflected by urinary nitrogen loss). This study was, therefore, designed to obtain base-line information concerning the metabolic and hormonal response to fasting in normal subjects and in two subjects with mild diabetes in the hope that such information would provide at least partial insight into some of these problems. In brief, we found in the normal subjects that the well-integrated release of peripheral fuels and the maintenance of blood glucose concentrations were probably related to insulin concentrations, suggesting but not necessarily proving that insulin is the primary signal responsible for fuel control during starvation. The studies also suggested that glucose metabolism, particularly by brain, must be decreased in order for man to survive prolonged periods of caloric deprivation.

Methods

Subjects. Six normal male subjects were selected to provide a diverse spectrum of body size and shape (Table I). Five (N_1, N_3, N_4, N_5, and N_6) were divinity students, and the sixth (N_2) was a sporting-goods salesman. All were in perfect health and had been consuming an average diet estimated to contain over 250 g of carbohydrate and 80 g of protein with variable amounts of fat. Subjects N_2 and N_4 were intentionally selected because of a family history of diabetes; their mothers had maturity-onset diabetes and required insulin. Since both of these subjects were indistinguishable in all respects from

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TABLE I

Clinical data for normal and diabetic subjects

Subject	Figure symbol	Age	Weight		Height	BSA		Ke*	Per cent deviation from population mean weight†
			Initial	Final		Initial	Final		
		years	*kg*		*cm*	*m²*		*mEq*	
Normals									
N_1	○	26	77.5	72.0	169	1.87	1.81	3,391	+17
N_2	×	39	87.0	78.8	181	2.08	2.00		+10
N_3	□	24	75.4	68.5	181	1.97	1.88	3,989	− 4
N_4	△	22	94.6	88.8	181	2.18	2.10	4,330	+24
N_5	●	25	63.5	58.2	166	1.72	1.66	3,162	− 5
N_6	⊙	25	72.7	67.2	174	1.89	1.82	3,402	0
Diabetics									
D_1	+	37	75.6	70.9	185	2.00	1.95		−12
D_2	▲	39	94.0	89.3	174	2.09	2.05		+20

* Equilibration pool of ^{42}K, 48 hours.
† From Metropolitan Life Insurance tables, 1959.

the other four individuals, they were included in the normal group.

The two diabetic subjects were chosen for their relatively young age, their willingness to undergo the study, and for their type of diabetes, which was maturity-onset but still sufficient in degree to make the diagnosis indubitable. One year before the study, subject D_1, a business manager, had experienced 2 months of fatigue and a 25-pound weight loss associated with polydypsia and polyuria. He received insulin, 20 to 40 U NPH, for 3 weeks and was then given tolbutamide, 0.5 g by mouth twice daily, until 4 days before this study, when treatment was terminated. Subject D_2, a contractor, had experienced polydypsia and polyuria 8 months before the study and received one single injection of 20 U of 40 U per ml Lente insulin, followed by 1 g of tolbutamide by mouth for 1 month. Then, like subject D_1, he followed a diet of 200 g carbohydrate, 90 g protein, and 70 g fat. Both diabetic subjects had experienced transient glycosuria but only after ingesting amounts of carbohydrate beyond their dietary allowances.

All subjects had normal hematological and urine analyses in addition to normal chest and abdominal radiographic examinations, electrocardiograms, thyroid indexes, serum enzymes (alkaline phosphatase, lactic dehydrogenase, glutamic-oxaloacetic transaminase), serum proteins (electrophoresis), and serum lipids and lipoproteins (electrophoresis and chemical analyses for total lipids, triglycerides, phospholipids, cholesterol, and cholesterol esters).

Blood and urine collection. Samples were collected according to the schedule given in Table II.

Each morning at 8:00 a.m., after the subject had been resting for 30 minutes, blood was obtained from either of the two major antecubital veins (cephalic or median cubital) without stasis, and when, on occasion, a tourniquet was needed, it was released for 10 to 20 seconds before taking the sample. The needle was placed well up the vein proximally, and the samples thus represented mixed deep and superficial venous drainage. Thirty-five ml of blood was withdrawn, portions were added to oxalate-fluoride for glucose and free fatty acid analyses, and the remainder was allowed to clot. Fifteen ml of blood was then rapidly drawn into a syringe from which it was immediately injected into 15 ml of iced 1 M perchloric acid. The deproteinized supernatant fluid was frozen and later used for acetoacetate, β-hydroxybutyrate, glycerol, pyruvate, and lactate analyses. Whole blood samples in oxalate-fluoride and plasma and serum specimens were frozen and stored at − 20° C for later analysis.

Urine was collected in plastic containers immersed in ice. At 8 a.m., the end of each 24-hour period, the subject voided, the total volume was measured, and iced

TABLE II

Schedule of blood and urine collection

Day	Blood sample*	Urine collection	Calories	Intake H_2O	NaCl
				ml	*mEq*
1	0 1	1	ivGTT† Lunch Supper	Ad libitum	Ad libitum
2		2	0	1,500	0
3	2	3	0	1,500	0
4	3	4	0	1,500	0
5	4	5	0	1,500	0
6	5	6	0	1,500	17
7	6	7	0	1,500	17
8	7	8	0	1,500	17
9	8		ivGTT Lunch Supper	Ad libitum	Ad libitum

* All blood samples were taken at 8 a.m.
† ivGTT = intravenous glucose tolerance test.

distilled water was added to make 2 L. Portions of this were removed and frozen for later analyses. To increase the accuracy of the terminal void in each collection, we gave subjects the last 200 to 400 ml of their 24-hour water ration 1 to 2 hours before the 8 a.m. collection time. Glucose tolerance tests were performed by the rapid iv injection of glucose (0.5 g per kg body weight) in 2 to 4 minutes with blood samples at appropriate intervals timed from the end of the infusion.

Chemical analyses. Glucose was measured in both blood and plasma by the Somogyi-Nelson technique in duplicate (16, 17), by glucose oxidase (18), and by the Technicon Autoanalyzer ferricyanide method. Appropriate corrections were made for red cell stromal and protein volumes (19, 20). Each value in the Figures and Tables represents the mean of all four determinations. In those instances in which a value varied by 5% or more of the mean, all the analyses were repeated. Free fatty acids in plasma were measured by the Trout, Estes, and Friedberg modification (21) of the method of Dole (5). Glycerol and pyruvic and lactic acids in the potassium bicarbonate-neutralized perchlorate supernatant fluid were analyzed by enzymatic techniques (22). All enzymes and cofactors were obtained commercially.[1] Creatinine, uric acid, and urea in serum were determined by the Technicon Autoanalyzer and serum CO_2 by the Van Slyke standard manometric analysis.

β-Hydroxybutyrate and acetoacetate were measured enzymatically by the method of Williamson, Mellanby, and Krebs (23) partially following the modifications suggested in the Boehringer commercial pamphlet of 1964. The supernatant fluid of the perchloric acid-treated blood was filtered in the cold and 5 ml used for β-hydroxybutyrate determination after appropriate dilution. This was mixed with 0.65 ml of a solution containing 3 M KOH and 1 M K_2CO_3, and to this was added 0.3 ml of 0.025 M β-NAD. After standing at 20° to 25° C for 30 minutes, the mixture was centrifuged and 3.5 ml of the supernatant fluid (pH 9.5) placed in a cuvette. The initial optical density was determined at 340 mμ; then 0.02 ml of a suspension of β-hydroxybutyric dehydrogenase (5 mg per ml) was added and the reaction allowed to go to completion in a water bath at 37° C. The optical density was redetermined at 45 minutes, and appropriate calculations were applied to determine the concentration of β-hydroxybutyrate present in the original sample. For the acetoacetate determinations, 5 ml of the deproteinized blood filtrate, appropriately diluted, was mixed with 0.45 ml of 3 M K_3PO_4. After 30 minutes at 20 to 25° C the mixture was centrifuged and 3.5 ml (pH 7) placed in a cuvette. To this, 0.1 ml of β-NADH was added and the initial optical density determined at 340 mμ; then 0.02 ml of β-hydroxybutyric dehydrogenase suspension (5 mg per ml) was added and the optical density redetermined after 25 minutes at 20 to 25° C, at which time the reaction had gone to completion. Appropriate blanks and standards were run for both substrates with each set of determinations. Mean recovery of β-hydroxybutyrate added to blood and carried through the entire procedure was 91.4 ± 5.4 (SD). Similarly, recovery of acetoacetate was 84% ± 5.5 (SD). The values presented in the Tables were not corrected for this loss.

Serum immunoreactive insulin was measured by a modification (24) of the double antibody technique of Morgan and Lazarow (25) and growth hormone by both a double antibody technique (26) and by the method of Glick, Roth, Yalow, and Berson (27). Insulin values are the means of duplicate analyses that agreed within 10%. Growth hormone was initially assayed in duplicate by the double antibody system of Schalch and Parker (26) and, if these values were less than 2 mμg per ml, were run in triplicate with the chromatoelectrophoretic method (27). For both systems, the values reported are the means of duplicate or triplicate analyses that agreed within 10%. Serum insulin-like activity was determined by the method of Renold and co-workers (28), in which oxidation of glucose-1-^{14}C to $^{14}CO_2$ in the presence of surviving isolated rat epididymal adipose tissue is used as an index of activity. Each assay was monitored statistically (29) and the index of precision determined; the corresponding lambda values were 0.10, 0.11, 0.16, and 0.21. Serum was diluted before assay to a 25% concentration with buffer, and the results are corrected for this dilution.

Nitrogen in urine was determined in duplicate by the standard Kjeldahl technique in two laboratories. It was repeated if any single value varied by greater than 10% of the mean. Urinary acetoacetate and β-hydroxybutyrate concentrations were measured in the same manner as in whole blood. Creatinine and uric acid in urine were determined by the Technicon Autoanalyzer and sodium and potassium by indirect flame photometry. Urinary 17-hydroxycorticoids were determined by the method of Reddy (30) and ketosteroids by that of Drekter and associates (31).

Glucose turnover studies. The glucose turnover studies, performed at the end of the fast in all eight subjects and also at the beginning in the two diabetics, were done according to methods and calculations previously described (11, 12). Twenty μc (1.71 mg) of glucose-1-^{14}C [2] in 2 ml of saline was injected into the tubing of an isotonic saline infusion within 60 seconds. At intervals of 30 minutes for 3 hours, blood samples were withdrawn and immediately deproteinized according to the method of Somogyi (17). Separation of glucose from acidic components of the protein-free filtrate and oxidation with periodic acid were accomplished as previously described (11, 12). The CO_2 derived from carbons 1 to 5 of the glucose was isolated as $BaCO_3$ and counted as a suspension in a standard 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP)-toluene phosphor solution containing thyxin in a concentration of 30 g per L. The formaldehyde, derived from carbon 6 of the glucose, was isolated as the formaldimedone and counted as such in the POPOP-toluene phosphor solution, in which it is completely soluble. All counting was done in a Packard

[1] Boehringer, Mannheim, Germany.

[2] New England Nuclear Corp., Boston, Mass.

liquid scintillation spectrometer, model 314-DC. Calculations were performed on an IBM 1410 computer (12).

Calorimetry. Total energy balance was determined during the entire fasting period in subjects N_1 and N_6 and in the two diabetic subjects, D_1 and D_2. In the other four subjects (N_2 to N_5), this procedure was performed only on days 6, 7, and 8. The technique was conventional indirect calorimetry, as modified for clinical application by Kinney (32). Expired air was collected for 5-minute periods every hour of the waking day with the subject reclining in bed without specified previous restriction of activity. Samples, stored temporarily for 5 to 15 minutes in aluminized plastic bags, were run through an analysis train composed of an American Meter Co. wet test flowmeter (model 804), a modified Liston-Becker carbon dioxide analyzer (model 16), and a Beckman E2 oxygen analyzer. Analyzers were standardized with known gas mixtures before each run.

The respiratory artifact associated with sampling, usually hyperventilation, is recognizable by RQ variations from sample to sample; a preliminary 24-hour period was allowed for acclimatization to the mask, and the results were discarded. After this period, as Kinney has shown (32), mean caloric expenditure so determined accounts for 90% or more of the measured caloric intake in a subject under relatively steady state conditions.

The patients' activities were not intentionally restricted at any time except before the morning phlebotomy; however, it was noted that they restricted their activities to dressing in casual clothes each morning after their weight had been taken and to meeting usual hygienic needs in addition to limited ambulation about the Clinical Research Center. Fluid intake was 1,500 ml water per day, and for the last 3 days of the study, the subjects received 17 mEq of NaCl to compensate partly for the volume depletion from blood sampling.

Each subject was fully advised as to the nature and extent of the study before giving consent and being accepted.

Results

Normal base-line fasting values. In Table III are summarized the mean values of all constituents measured in plasma (or serum). Samples 0 and 1 are values after a routine overnight fast. Samples 2 to 8 are for each succeeding day of total fast (180 hours). Glucose concentrations reached a plateau by the third day of fast and remained extremely stable thereafter (Figure 1) as did the levels of immunoreactive insulin (Figure 2). The correlation coefficients of the nine paired values of insulin and glucose for each normal subject showed probability values less than 0.01 in three, and less than 0.001 in three. All paired values of insulin and glucose when combined for all normal subjects had a correlation coefficient of 0.604 ($p < 0.001$). A linear regression was derived ($y = 57.2 + 1.22\,x$) with the y intercept, 57.2 mg per 100 ml, and the 95% confidence limits of this value were ± 4.64.

In contrast to serum insulin measured by the immunoassay, serum insulin-like activity, as determined on rat adipose tissue, exhibited a dif-

TABLE III

Serum or plasma concentrations in the six normal subjects

	Days of study								
	0	1	2	3	4	5	6	7	8
Glucose, *mg/100 ml*	77 ± 2*	84 ± 1	73 ± 2	68 ± 2	65 ± 1	66 ± 1	62 ± 2	64 ± 1	63 ± 1
FFA, *mmoles/L*	0.53 ±0.02	0.42 ±0.04	0.82 ±0.08	1.04 ±0.07	1.15 ±0.11	1.27 ±0.07	1.18 ±0.05	1.19 ±0.05	1.88 ±0.05
Acetoacetate, *mmoles/L*		0.013 ±0.003	0.16 ±0.03	0.51 ±0.07	0.65 ±0.10	0.77 ±0.09	0.85 ±0.08	0.95 ±0.08	1.09 ±0.11
β-Hydroxybutyrate, *mmoles/L*		0.016 ±0.001	0.39 ±0.11	1.64 ±0.14	2.24 ±0.12	2.87 ±0.23	3.13 ±0.24	3.58 ±0.26	4.23 ±0.34
Glycerol, *μmoles/L*		62 ± 6	86 ± 12	95 ± 14	91 ± 10	100 ± 15	76 ± 10	80 ± 10	164 ± 41
CO_2 content, *mmoles/L*	23.8 ±0.8	25.5 ±1.0	23.7 ±1.1	20.5 ±1.3	20.7 ±1.3	20.2 ±1.1	19.4 ±1.1	20.7 ±1.0	18.0 ±1.1
Urea nitrogen, *mg/100 ml*	12.2 ±0.7	13.0 ±1.1	14.0 ±1.4	16.2 ±1.4	16.2 ±1.6	14.3 ±1.0	13.2 ±1.1	13.0 ±1.3	13.3 ±1.6
Uric acid, *mg/100 ml*	6.2 ±0.4	6.2 ±0.7	7.7 ±0.5	9.2 ±0.9	11.6 ±1.2	12.1 ±0.9	13.5 ±0.9	13.8 ±1.1	15.0 ±0.5
Creatinine, *mg/100 ml*	1.02 ±0.10	0.90 ±0	1.05 ±0.16	1.10 ±0.20	1.20 ±0.15	1.10 ±0.08	1.13 ±0.08	1.02 ±0.10	1.20 ±0.07
Insulin, *μU/ml*	14.0 ±1.8	15.2 ±2.3	9.2 ±0.8	8.0 ±0.7	7.7 ±0.4	8.6 ±0.8	7.7 ±0.6	7.7 ±0.5	8.3 ±1.0
Growth hormone, *mμg/ml*	0.3 ±0.3	1.9 ±1.2	3.1 ±1.9	5.8 ±1.6	3.7 ±1.0	8.8 ±2.9	6.0 ±2.9	2.5 ±1.2	3.4 ±1.3

* ± Standard error of the mean.

ferent behavior. Over the fasting period, four subjects showed an increase, one no change, and one a decrease in activity. The mean value, corrected for the 1:4 dilution in the assay, increased from 372 to 432 μU per ml, and this difference was statistically significant ($p < 0.05$) by the paired t test. The initial value after an overnight fast was well within the range observed in 56 control subjects: mean $\pm$ SEM = 364 $\pm$ 28 μU per ml (33).

Free fatty acids achieved a plateau by the third day, except for a significant increase on the last day, probably due to a subjective response to insertion of an indwelling needle for the glucose turnover study. That this increase in fatty acid concentration was due to an increased lipolysis in adipose tissue, probably as a result of adrenergic stimulation, is supported by the parallel increase in blood glycerol. Acetoacetate and β-hydroxybutyrate rose gradually throughout the study as did uric acid. The ratio of β-hydroxybutyrate to acetoacetate remained relatively constant after the second day of fast. However, when the levels were so low as to be barely detectable (first 2 days of the study), the ratio approached unity. As expected, the increase in organic acids (acetoacetic, β-hydroxybutyric, and free fatty acids) was accompanied by a corresponding decrease in [HCO_3^-] with their sum remaining constant within the errors of the methods (Figure 3).

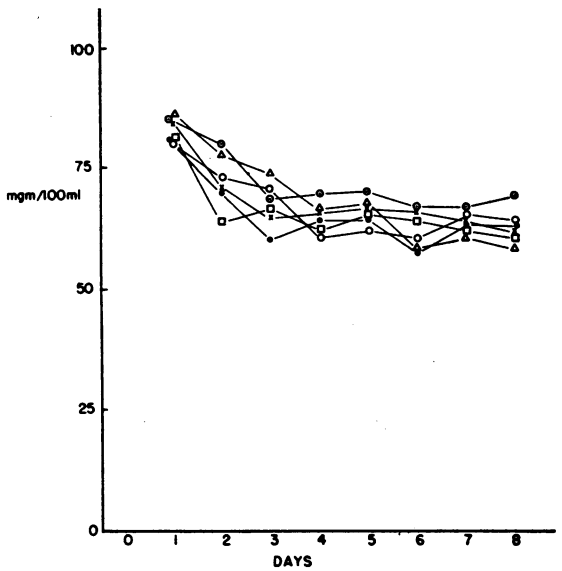

FIG. 1. INDIVIDUAL VALUES FOR PLASMA GLUCOSE FOR EACH NORMAL SUBJECT OVER 8-DAY PERIOD OF FAST.

Serum urea nitrogen exhibited a progressive rise and fall. As will be shown later, this was not due to altered renal function but to a rise and fall in nitrogen production and excretion. Venous lactic and pyruvic acid concentrations were measured throughout the study in subjects N_1 and N_6 and on the last 2 days of the study in all subjects. No change was noted throughout the fast (Figure 3), and the final mean lactate to pyru-

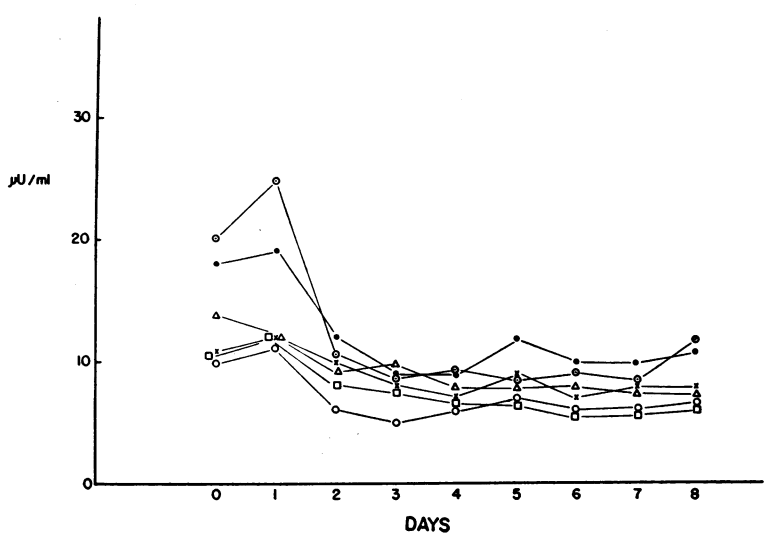

FIG. 2. INDIVIDUAL VALUES FOR SERUM IMMUNOREACTIVE INSULIN FOR EACH NORMAL SUBJECT ON EACH DAY OF FAST.

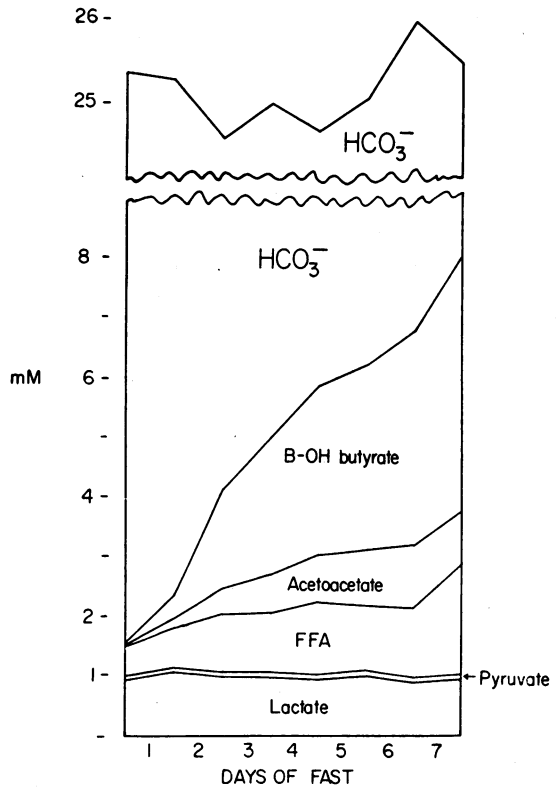

FIG. 3. THE SUM OF LACTIC, PYRUVIC, FREE FATTY, ACETOACETIC, AND β-HYDROXYBUTYRIC ACID CONCENTRATIONS AND THE RECIPROCAL DECREASE IN [HCO_3^-]. Mean of values from six normal subjects.

vate ratio was 9.1 ± SEM 0.2 (n = 18). Arterial values, although far more significant, were intentionally not obtained to minimize trauma and blood loss.

Finally, the mean values for growth hormone

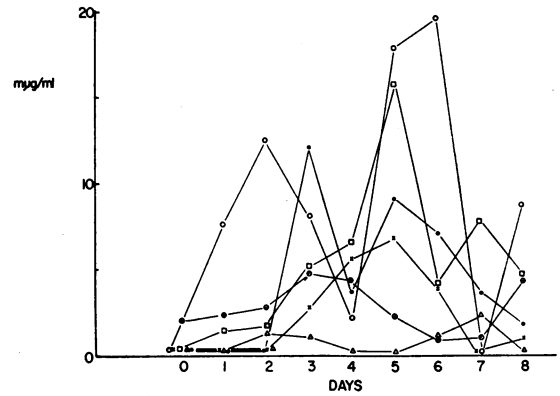

FIG. 4. SERUM GROWTH HORMONE CONCENTRATIONS IN SIX NORMAL SUBJECTS DURING 8 SUCCESSIVE DAYS OF FASTING.

exhibited a rise and fall; however, the individual values (Figure 4) were extremely variable. No correlation between growth hormone levels and free fatty acids, glucose, or insulin could be made. Likewise, there was no correlation between any of these parameters and the habitus of the individual or the presence or absence of a family history of diabetes (N_2 and N_4).

Base-line fasting values in diabetes. In Table IV are summarized the values derived from the two diabetic subjects. Values that are greater than 2 or 3 SD from the mean of the normals are appropriately marked. The diabetics, as expected, showed high fasting glucose values early in the study. Subject D_1 had abnormally low values on days 3 through 7; however, D_2 had abnormally elevated values on all but day 6. Both

TABLE IV

Plasma or serum concentrations

	Subject D_1: days of study								
	0	1	2	3	4	5	6	7	8
Glucose, *mg/100 ml*	98*	96*	69	57†	53†	47*	50*	55*	59
FFA, *mmoles/L*	1.09*	0.84*	1.13	1.84*	1.67†	1.40	1.42	1.90*	2.24†
Acetoacetate, *mmoles/L*	0.036*	0.040*	0.20	0.49	0.66	0.80	0.97	0.95	1.11
β-Hydroxybutyrate, *mmoles/L*	0.066*	0.054*	0.39	1.89	2.48	3.07	4.18	4.30	4.72
Glycerol, *μmoles/L*	62	39	55	111	96	51	112	103	144
CO_2 content, *mmoles/L*	25		28	25	24	22	22	21	21
Urea nitrogen, *mg/100 ml*	13	14	15	20	16	13		11	12
Uric acid, *mg/100 ml*	4.5		5.4	6.8	8.2	9.6	10.1	10.5	10.7*
Creatinine, *mg/100 ml*	1.0		1.0	1.0	1.0	1.1	1.1	1.1	1.1
Insulin, *μU/ml*	30*	33*	30*	27*	24*	24*	29*	26*	24*
Growth hormone, *mμg/ml*	3.3*	1.4	25.0*	14.8†	11.8*	12.0	21.0†	8.0	4.4

* > 3 SD from mean of normals.
† > 2 SD from mean of normals.

diabetics showed higher levels of acetoacetic and β-hydroxybutyric acids during the first 2 days.

Other sporadically abnormal values are noted, besides those for insulin and growth hormone. Chromatography of the sera after incubation with insulin-^{131}I failed to detect antibody (34). This was carefully excluded since both subjects had received insulin many months previously, and persistence of insulin antibody could have contributed to their high serum immunoreactive insulin levels. Both subjects also had abnormally elevated levels of growth hormone, but not persistently.

Intravenous glucose tolerance tests. Table V summarizes the data on glucose and free fatty acid levels in all subjects during iv glucose tolerance tests both before and after the fast. The well-known grossly abnormal tolerance is noted in all the normal subjects after the fast, whereas the diabetic subjects showed no change in tolerance, and after the fast were generally indistinguishable from the normals. The coefficient of glucose disappearance (K) was also calculated (35) and is listed. If we extrapolate to time 0 the plot derived from the logarithm of glucose concentration against time, the calculated glucose space for the normal subjects before the fast was 25.5 ± 0.9% of body weight and after the fast 27.4 ± 1.3%, an insignificant difference.

In Table VI are listed the concentrations of insulin and growth hormone during the tolerance tests before and after the fast. The normal subjects exhibited a brisk insulin response, achieving peak values at 1 minute after the end of the glucose infusion both before and, surprisingly, after the fast, but the magnitude of the response was much less in the latter. The individual values, plotted in Figure 5, show a fairly consistent pattern of response for each individual. The glucose load failed to elicit a definite insulin response both before and after the fast in subject D_1. D_2 showed a delayed and hyperactive response before the fast and an earlier but diminished response after the fast.

The correlation coefficient of all paired values of serum immunoreactive insulin and plasma glucose during the tolerance tests in the normal subjects before the fast was 0.685 ($p < 0.001$) and after fast was 0.634 ($p < 0.001$). Linear regression equations were calculated for the tests before and after the fast and are plotted in Figure 6. The bars indicate the 95% confidence limits of the respective intercepts on the plasma glucose axis. There was no significant difference in mean slopes; however, there was a significant difference ($p < 0.01$) between the glucose intercepts before (61 mg per 100 ml) and after (129 mg per 100 ml) the fast.

As noted in the daily fasting results, growth hormone responses during the tolerance tests were markedly variable. Individual values are illustrated in Figure 7. No consistent pattern was found among individuals, or before and after the fast, except for the sporadically higher values of the diabetics, particularly D_1 (Table VI). Also, an obvious correlation between patient discomfort and the growth hormone response could not be made.

TABLE IV

in the two diabetic subjects

Subject D_2: days of study								
0	1	2	3	4	5	6	7	8
107*	110*	100*	90*	80*	75*	70	72*	72†
0.95*	0.90*	0.95	1.20	1.37	1.44	1.42	1.54†	2.03†
0.029†	0.029†	0.082	0.22	0.46	0.40	0.78	0.71	0.77
0.058*	0.058*	0.21	0.81†	1.58†	1.55†	2.78	2.64	3.18
62	36†	36	54	41†	48	70	68	96
26	27	26	24	24	23	23	23	19
14	14	17	17	20	17	16	14	14
6.4	7.3	8.1	9.0	10.5	12.6	14.2	15.6	16.6
1.1	1.2	1.1	1.1	1.1	1.2	1.2	1.2	1.3
51*	55*	44*	36*	42*	46*	53*	45*	40*
<1.0	<1.0	<1.0	20	16.1*	32*	8	15.6*	2.0

TABLE V

Glucose tolerance tests

	Minutes after termination of infusion													Coefficient of glucose disappearance (K)
	0	1	3	5	10	20	30	40	50	60	90	120	180	
														%/min
Normals (n =6) (±SE)														
Before fast (day 0)														
Glucose, *mg/100 ml*	77±2	328±29	269±8	247±4	229±3	181±10	143±10	119±8	104±6	86±6	68±5	64±4	66±5	2.1±0.1
FFA, *mmole/L*	0.56 ±0.04				0.50 ±0.09	0.40 ±0.08				0.31 ±0.03				
After fast (day 8)														
Glucose, *mg/100 ml*	63±1	317±11	283±16	273±12	254±14	230±9	214±8	202±7	193±8	186±4	166±7	152±7	120±6	0.63±0.02
FFA, *mmoles/L*	2.01 ±0.09				1.47 ±0.12	1.22 ±0.08				0.79 ±0.07	0.76 ±0.05	0.92 ±0.06	1.17 ±0.08	
Diabetics														
D_1 Before fast (day 0)														
Glucose, *mg/100 ml*	98*	226	228†	235	225	199	188	186*	180*	169*	159*	124*	111*	0.52*
FFA, *mmole/L*	0.87*				0.95†	0.81†				0.75*		(0.66)‡	(0.54)	
After fast (day 8)														
Glucose, *mg/100 ml*	59	316	255	228	232	208	200	195	188	187	165	155	126	0.52
FFA, *mmoles/L*	1.63				1.27	1.13				0.83	1.13	1.12	0.96	
D_2 Before fast (day 0)														
Glucose, *mg/100 ml*	107*	301	283	271†	270*	238†	214†	202*	194*	187*	144*	124*	90†	0.54*
FFA, *mmole/L*	0.94*				0.87†	0.78†				0.46	(0.24)	(0.32)	(0.20)	
After fast (day 8)														
Glucose, *mg/100 ml*	72†	380†	327	325	310	285†	256†	233	227	224*	208*	188*	139	0.58
FFA, *mmoles/L*	2.43				2.35*	2.22*				1.21†	0.96	0.86	1.12	

* > 3 SD from mean of normals.
† > 2 SD from mean of normals.
‡ Values in parentheses were not compared to normals.

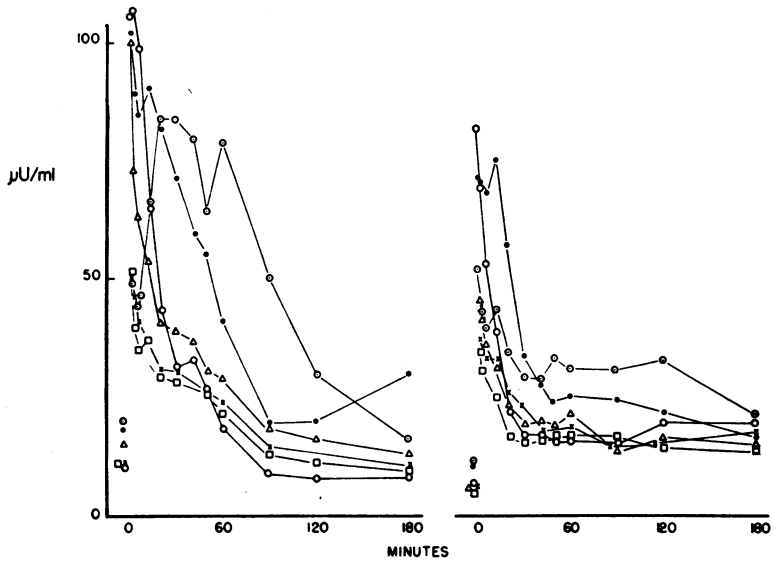

FIG. 5. INDIVIDUAL LEVELS OF SERUM IMMUNOREACTIVE INSULIN DURING IV GLUCOSE TOLERANCE IN EACH NORMAL SUBJECT BEFORE (LEFT) AND AFTER (RIGHT) FASTING.

Glucose turnover. In Table VII are listed the values from glucose turnover studies in the six normal subjects after the fast and in the two diabetics both before and after. Also included for comparison are the glucose spaces and pool sizes calculated with both glucose-^{14}C and the iv glucose tolerance test. The mean pool size of the normals, by isotope dilution, was 6.6 g per m^2 body surface; by the iv glucose tolerance, it was 6.7 g per m^2 body surface, a surprisingly good agreement. Glucose turnover was quite variable, subject N_3 having the highest and N_1 the lowest. Subject N_3 likewise had the highest rate of recycling of 33% or 41.2 g per m^2 per day (12). This same individual exhibited the highest absolute glucose turnover after subtraction of the amount recycled, 84 g per m^2 per day, and, as expected, the highest nitrogen loss (Figure 8).

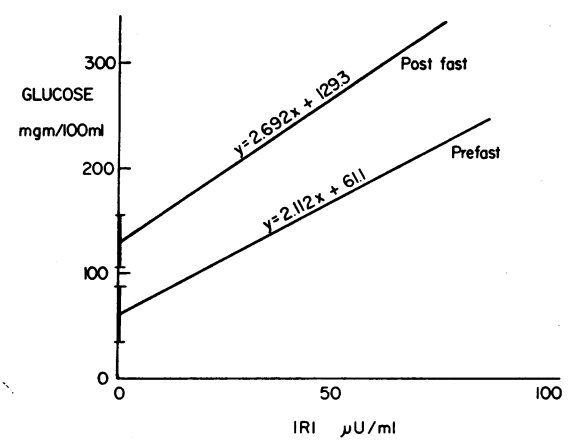

FIG. 6. LINEAR REGRESSION OF PLASMA GLUCOSE AND SERUM IMMUNOREACTIVE INSULIN (IRI) FOR ALL SIX IV GLUCOSE TOLERANCE TESTS IN NORMAL SUBJECTS BEFORE AND AFTER FASTING. Bars indicate the 95% confidence limits of respective intercepts on glucose axis.

The two diabetic subjects were indistinguishable from the normals after the fast except for the larger glucose pool in subject D_2. Their values before fasting compare to those described in mild diabetics with this technique (11).

Total energy balance. Table VIII summarizes the extensive analyses of metabolic balance on the last day in all eight subjects. In the two normal subjects who were followed throughout the study, 4 days was required for the nonprotein RQ to fall to 0.70, in agreement with the observations of Benedict on his single subject (2). In the two diabetics, the nonprotein RQ fell to 0.70 on the first day of the fast and remained at or below this level throughout the succeeding days.

The surprising agreement of total calories consumed based on surface area by the six normal subjects is probably fortuitous. The slightly higher energy consumed by D_1 and D_2 was per-

TABLE VI

Serum insulin and growth hormone

		Minutes				
		0	1	3	5	10
Before fast (day 0)						
Normals (n=6) ± SE	Insulin, $\mu U/ml$	14 ± 2	78 ± 13	68 ± 12	61 ± 10	58 ± 8
	GH, $m\mu g/ml$	0.3 ± 0.3	0 ± 0	0.23 ± 0.23	0.2 ± 0.2	1.4 ± 1.1
Diabetics						
D_1	Insulin, $\mu U/ml$	30*	26	25	23	27
	GH, $m\mu g/ml$	3.3	4.3*	2.8*	3.2*	3.1
D_2	Insulin, $\mu U/ml$	51*	51	52	51	69
	GH, $m\mu g/ml$	<1.0	<1.0	<1.0	<1.0	2.0
After fast (day 8)						
Normals (n=6) ± SE	Insulin, $\mu U/ml$	8 ± 1	54 ± 8	48 ± 7	43 ± 6	41 ± 7
	GH, $m\mu g/ml$	3.4 ± 1.3	2.6 ± 0.5	2.0 ± 0.7	0.9 ± 0.4	1.6 ± 0.7
Diabetics						
D_1	Insulin, $\mu U/ml$	24*	39	35	33	34
	GH, $m\mu g/ml$	4.4	2.0	2.0	1.6	2.2
D_2	Insulin, $\mu U/ml$	40*	64	68	67	96*
	GH, $m\mu g/ml$	2.0	<1.0	<1.0	<1.0	<1.0

* > 3 SD from mean of normals.
† > 2 SD from mean of normals.

haps due to a greater degree of activity and, although significantly different from the normals, is probably of little import. The same is true of the lower RQ on the last day of study. If the distribution of calories is calculated from the nitrogen loss and the nonprotein RQ, only N_5 expended any carbohydrate, and again, the error of the method precludes any significance to this

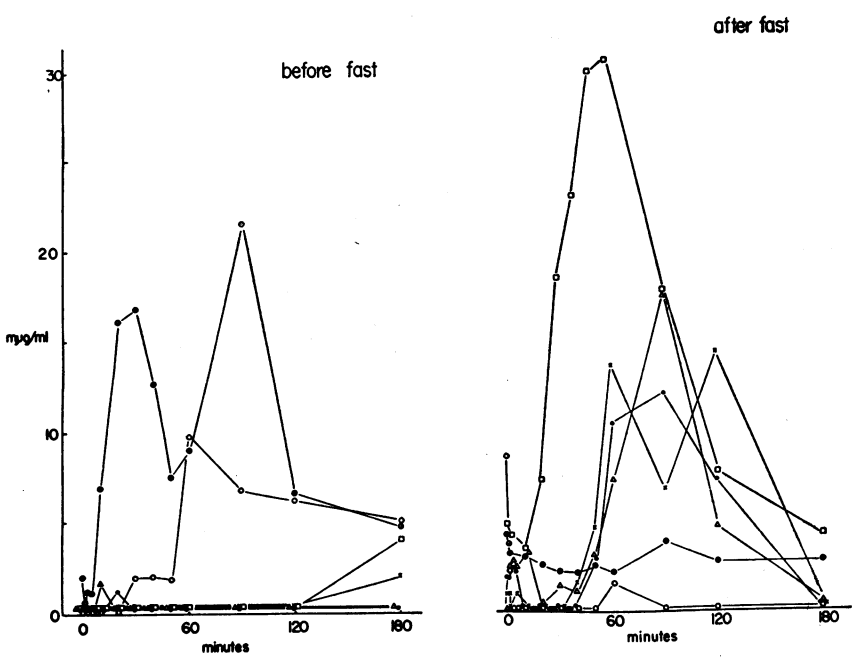

FIG. 7. CONCENTRATIONS OF GROWTH HORMONE IN SERUM IN EACH NORMAL SUBJECT DURING GLUCOSE TOLERANCE TESTS BEFORE AND AFTER THE PROLONGED FAST.

TABLE VI

(GH) levels: ivGTT before and after fast

Minutes							
20	30	40	50	60	90	120	180
52 ± 10	48 ± 10	42 ± 10	39 ± 7	36 ± 9	21 ± 6	16 ± 4	15 ± 3
2.9 ± 2.6	3.1 ± 2.8	2.5 ± 2.1	1.5 ± 1.2	3.1 ± 2.0	4.7 ± 3.5	2.1 ± 1.3	2.6 ± 0.9
30	31	31	33	31	31	34	38†
1.4	<1.0	4.4	13.6*	18.4*	18.4	15.0*	1.0
98	102	100†	100*	106*	115*	102*	68*
17.0†	10.0	7.6†	6.4	2.0	<1.0	<1.0	<1.0
30 ± 6	23 ± 3	21 ± 2	21 ± 3	22 ± 2	20 ± 3	20 ± 3	17 ± 1
1.6 ± 1.2	3.7 ± 3.0	4.7 ± 3.7	7.2 ± 4.6	10.9 ± 4.4	9.7 ± 3.0	6.1 ± 2.0	1.2 ± 0.7
31	27	29	30	28	31	32	33*
3.8	25.4†	32.2*	39.0†	22.0	16.0	8.4	0
80*	71*	70*	63*	64*	86*	71*	82*
<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

observation. Nevertheless, knowing the approximate contribution of lipid to the fuel consumed and assuming that glycerol comprises about 10% of lipid weight, one can calculate the glycerol mobilized from adipose depots. Since this moiety can be readily incorporated into glucose, the nonglycerol synthesis of glucose can be calculated from the value obtained from Table VII, namely, the glucose turnover minus the amount recycled. The mean is 55 ± 6 g per m² per day, and the two diabetic subjects fall well within the normal range.

Urinary excretion. Components measured in the urine are summarized in Table IX. Nitrogen excretion (Figure 8) rose from the second or third day, peaked on the fourth day, and then declined, parallel to the rise and fall in serum urea nitrogen (Table III). Creatinine excretion

TABLE VII

Glucose metabolism (day 8)

	Glucose pool				Glucose space		Turnover			Recycle			[Turnover-recycle]
	^{14}C	ivGTT	^{14}C	ivGTT	^{14}C	ivGTT							
	g		*g/m²*		*% body wt*		*%/min*	*mg/kg/hr*	*g/m²/day*	*%*	*mg/kg/hr*	*g/m²/day*	*g/m²/day*
Normals (after fast)													
N_1	9.9	12.3	5.5	6.8	24.6	27.0	0.799	67.1	63	10.0	6.7	6.3	57
N_2	11.1	11.3	5.5	5.6	24.7	23.0	0.978	82.2	78	16.0	13.2	12.5	66
N_3	14.4	13.7	7.7	7.7	39.6	30.5	1.136	143.0	125	33.0	47.2	41.2	84
N_4	16.8	13.6	8.0	6.5	35.0	26.4	0.795	90.6	82	32.0	29.0	29.5	63
N_5	11.3	11.6	6.8	7.0	32.8	31.6	0.849	96.8	83	19.0	18.4	15.8	67
N_6	11.3	11.8	6.2	6.5	33.6	25.6	0.760	77.5	68	26.0	20.2	17.7	50
Mean			6.6	6.7	31.7	27.4	0.886	92.9	85	22.7	22.5	20.5	64
SE			±0.5	±0.2	±2.5	±1.3	±0.059	±10.9	±9	±3.7	±5.8	±5.2	±4.7
Diabetics													
D_1 (before fast)	33.1	21.8	16.5	10.9	45.6	34.4	0.726	192.9	173	15.0	28.8	26.0	147
D_1 (after fast)	15.5	13.0	8.0	6.7	35.8	31.2	0.787	103.9	90	22.0	22.9	19.8	70
D_2 (before fast)	36.8	22.6	17.5	10.8	32.3	26.6	0.713	167.0	180	28.0	46.8	50.3	130
D_2 (after fast)	21.0	13.5	11.3*	6.6	31.4	21.2	0.616	88.7	91	32.0	28.4	29.1	62

* > 3 SD from mean of normals (after fast). No values were found between 2 and 3 SD from the mean of normals.

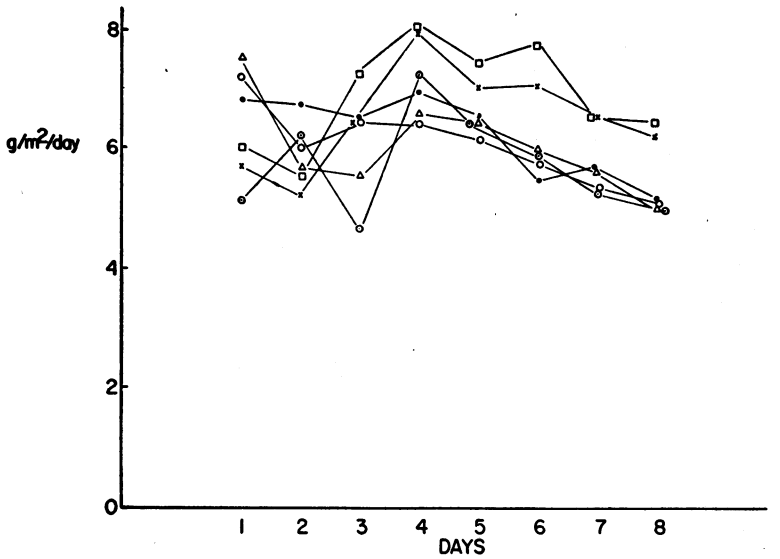

FIG. 8. URINARY NITROGEN EXCRETION IN NORMAL SUBJECTS DURING TOTAL FASTING. There is a significant increase on days 4 and 5 in all subjects when compared to days 2 and 8.

TABLE VIII

Energy metabolism (day 8)

	Total calories	Nonprotein respiratory quotient	Caloric distribution			Estimated glycerol	Glucose [turnover-recycle]	Nonglycerol glucose synthesis
			Fat	Carbohydrate	Protein			
						g/m²/day	*g/m²/day*	*g/m²/day*
Normals								
N_1	1,160	0.705	1,003	0	137	10.7	57	46
N_2	1,110	0.700	922	0	180	9.8	66	56
N_3	1,100	0.656	940	0	178	10.0	84	74
N_4	1,100	0.705	975	0	135	10.4	63	53
N_5	1,040	0.730	810	74	139	8.6	67	58
N_6	1,100	0.706	980	0	134	10.4	50	40
Mean ± SE	1,100 ± 16	0.700 ± 0.009	938 ± 27	12 ± 11	150 ± 9	10.0 ± 0.3	64 ± 5	55 ± 6
Diabetics								
D_1	1,210	0.64	1,070	0	136	11.4	70	59
D_2	1,200	0.64	1,060	0	145	11.3	62	51

TABLE IX

Urinary excretion in the six normal subjects

	Days							
	1	2	3	4	5	6	7	8
				/m²/day				
Nitrogen, *g*	6.4 ± 0.32	5.9 ± 0.25	6.1 ± 0.43	7.2 ± 0.28	6.6 ± 0.35	6.3 ± 0.37	5.7 ± 0.35	5.5 ± 0.30
Creatinine, *mg*	948 ± 43	935 ± 16	875 ± 44	942 ± 18	902 ± 18	912 ± 15	898 ± 25	885 ± 24
Uric acid, *mg*	422 ± 23	318 ± 19	176 ± 32	112 ± 20	102 ± 16	134 ± 16	153 ± 20	136 ± 16
β-Hydroxybutyrate, *mmoles*	0.014 ± 0.010	0.12 ± 0.07	6.6 ± 2.5	19.3 ± 4.9	30.2 ± 6.7	37.8 ± 6.7	38.8 ± 7.8	41.0 ± 5.9
Acetoacetate, *mmoles*	0.025 ± 0	0.27 ± 0.09	1.67 ± 0.28	5.4 ± 0.97	5.4 ± 0.8	5.9 ± 0.7	5.3 ± 0.9	5.8 ± 0.4
Sodium, *mmoles*	82 ± 11	49 ± 9	37 ± 7	30 ± 9	19 ± 4	11 ± 2	7 ± 1	6 ± 1
Potassium, *mmoles*	37 ± 5	32 ± 1	20 ± 1	25 ± 1	27 ± 2	25 ± 1	22 ± 1	21 ± 1
17-Hydroxycorticoids, *mg*	3.3 ± 0.06	3.2 ± 0.04	3.7 ± 0.5	3.6 ± 0.3	3.6 ± 0.3	3.3 ± 0.2	3.2 ± 0.4	2.8 ± 0.2
17-Ketosteroids, *mg*	8.9 ± 1.7	10.2 ± 2.5	7.6 ± 1.4	6.1 ± 1.1	5.8 ± 1.1	5.5 ± 1.3	5.1 ± 1.2	4.6 ± 1.0

TABLE X

Urinary clearances in the six normal subjects

	Days							
	1	2	3	4	5	6	7	8
	L plasma/m²/day							
Urea	48 ± 5	42 ± 2	43 ± 2	46 ± 2	42 ± 3	44 ± 1	42 ± 1	37 ± 1
Creatinine	94 ± 6	93 ± 4	87 ± 7	89 ± 6	82 ± 6	83 ± 6	87 ± 10	76 ± 7
Uric acid	6.7 ± 0.4	4.4 ± 0.2	2.1 ± 0.3	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
β-Hydroxybutyrate	1.0 ± 1.2	0.8 ± 0.4	5.8 ± 0.2	9.3 ± 2.0	11.0 ± 2.0	12.4 ± 2.0	11.3 ± 2.0	11.0 ± 1.4
Acetoacetate	0.7 ± 0.5	3.0 ± 1.0	5.2 ± 0.9	9.3 ± 1.2	7.3 ± 0.9	7.2 ± 0.5	5.8 ± 0.8	5.7 ± 0.6

declined slightly. β-Hydroxybutyrate and acetoacetate increased as expected. It is noteworthy that the final ratio of β-hydroxybutyrate to acetoacetate was greater than that in serum. Sodium and potassium excretion, particularly the former, decreased in spite of replacement of 8 to 9 mEq sodium per m² per day during the last 3 days. This decrease was undoubtedly due to volume depletion accentuated by the daily phlebotomy. Urinary potassium showed a transient increase parallel to the rise and fall in nitrogen loss, but 1 day later.

Table X summarizes clearance data for each 24-hour period using the mean of the initial and final plasma level for calculation. Urea and creatinine clearances were essentially unchanged, except for the final day when there was a decrease in both. As mentioned above, this was probably due to volume depletion secondary to blood sampling. Uric acid clearance fell for 3 days then remained stable. These data agree closely with those of Goldfinger, Klinenberg, and Seegmiller (36), who measured urate clearance after infusion of acetoacetate or β-hydroxybutyrate.

Excretion data on the two diabetic subjects are

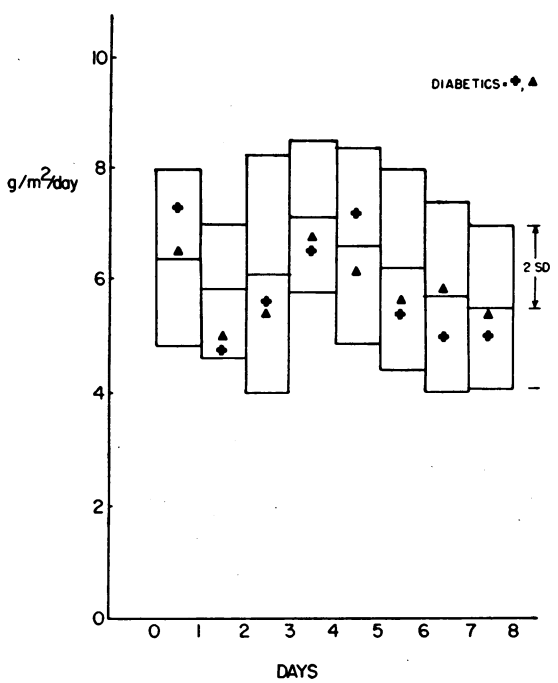

FIG. 9. URINARY NITROGEN EXCRETION OF THE TWO DIABETIC SUBJECTS (▲ AND ✤) SUPERIMPOSED ON THE MEAN VALUES OF THE SIX NORMAL SUBJECTS ± 2 SD OF THE MEAN.

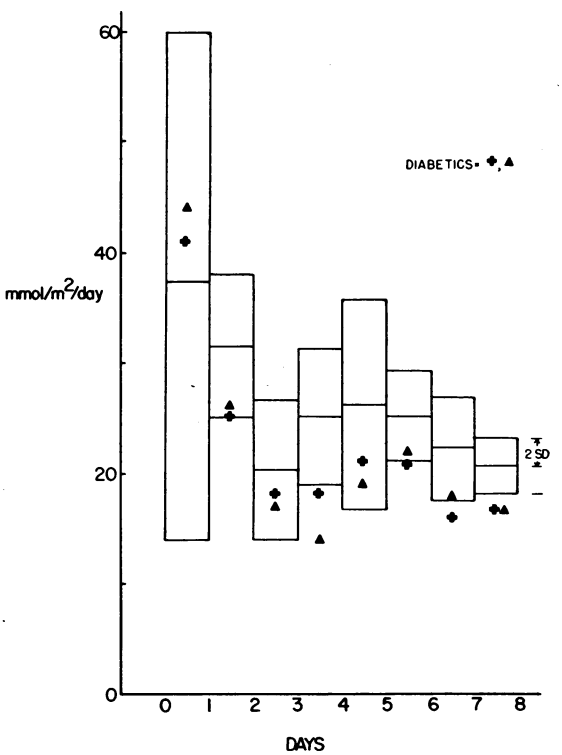

FIG. 10. URINARY POTASSIUM EXCRETION OF THE TWO DIABETIC SUBJECTS SUPERIMPOSED ON THE MEAN VALUES OF SIX NORMAL SUBJECTS ± 2 SD OF THE MEAN.

essentially indistinguishable from the normal subjects except for a slightly greater degree of ketonuria on day 1, in agreement with the higher blood ketone levels. In Figures 9 and 10 are plotted mean urinary nitrogen and potassium data ± 2 SD for the normals with values for D_1 and D_2. The diabetics appeared to conserve these two moieties to a greater degree than did the normals, but the same increases and decreases were noted as in the normal subjects. No significance can be presently attached to these differences due to the few subjects studied.

Discussion [3]

Insulin and glucose. Several studies have indicated that immunoreactive insulin is either unchanged, unmeasurable, or present in very low concentrations in the circulation of fasting man (37–41). That low, but physiologically significant, concentrations of insulin are present is suggested by experiments in animals in which the administration of insulin antibody (42, 43) or acute pancreatectomy (44) induced marked hyperglycemia, ketoacidosis, and death. In the present study, the assay was specifically manipulated to produce optimal discrimination at insulin concentrations in the vicinity of 10 μU per ml; hence, the low but strikingly uniform insulin levels in the six subjects and the close correlation between insulin and glucose levels strongly suggest the existence of a finely regulated metabolic system. One may also conclude that the low level of insulin in all likelihood does not modulate glucose concentration by regulating outflow to the tissues, since glucose turnover studies indicate that nearly all the glucose produced is probably utilized by tissues not sensitive to insulin, viz., brain (45, 46).

If we, therefore, accept the view that the role of insulin during a fast is to modulate the inflow of glucose into the bloodstream, we must then ask how insulin accomplishes this. It has been demonstrated in both experimental animals and man that the continuous *in vivo* infusion of small physiologic amounts of insulin causes a diminished inflow of glucose into the blood (47–51). Whether this represents a direct or indirect effect on the liver is another problem. Experiments using perfused liver preparations or slices have been inconsistent in demonstrating an effect of insulin on glucose utilization or production at physiological insulin concentrations (47). The recent finding of a hepatic glucose phosphotransferase, glucokinase, whose activity increases after insulin administration or carbohydrate feeding and decreases when insulin is deficient or lacking (52–54) also suggests that insulin may directly affect the liver. This enzyme, however, seems to have negligible activity in certain species, including humans (55).

The evidence pointing to an indirect effect of insulin on the liver has been more substantial. Several investigators have shown that increasing the concentration of either amino acid or lactic acid to levels well above those achieved *in vivo* (56, 57) or increasing free fatty acid (58–60) in the medium perfusing an isolated rat liver causes an almost immediate increase in net glucose production. This suggests that fuel presentation rather than a direct effect of insulin may be a more important factor in minute-to-minute glucose production. This hypothesis was clearly reviewed and enforced by Levine and Fritz 10 years ago (61).

Evidence that insulin, in the concentrations found during a prolonged fast, may control the flow of these peripherally stored fuels to the liver is quite strong. Fain, Kovacev, and Scow (62) and Kipnis (63) have recently demonstrated the exquisite sensitivity of adipose tissue lipolytic activity to insulin by showing that concentrations in the range of 1 μU per ml can inhibit the release of free fatty acids and glycerol from adipose tissue stimulated by various hormones. In addition, Reichard has obtained this effect with concentrations of insulin even below this subphysiologic level (64). In a similar way, Manchester and Young (65) demonstrated that the balance of amino acid and muscle protein is altered by a concentration of insulin close to that found in man by the immunoassay technique. More recently Smith and Long (66) have directed attention to the role of the interplay of insulin and glucocorticoids in the control of levels of circulating amino acids. In summary, it appears that during fasting a low but finely regulated

[3] The authors have chosen to discuss only some aspects of this study in order to conserve space and to limit the already lengthy bibliography.

concentration of insulin may modulate substrate delivery to the liver and thus indirectly the concentration of glucose in circulating fluids. The significant correlation coefficients and the linear regression of glucose and insulin concentrations support this hypothesis. Simply, a fall in blood glucose due to utilization by glucose-dependent tissues would be associated with a decrease in insulin, and this, in turn, would result in release of free fatty acid from adipose tissue and perhaps amino acid from muscle. Increased gluconeogenesis by liver would result, followed by replenishment of blood glucose and then an increase in insulin. Finally, the decrease in insulin would shut off the release of fuel from the peripheral depots, and the feedback loop would thus be completed.

Growth hormone. The finding of an increased level of growth hormone during fasting has been well documented (41, 67–70) and was again corroborated by this study. Of interest, however, was the great variability in growth hormone levels, some subjects showing no elevation and others a marked elevation during the fast. The individual responses after an iv glucose infusion both before and after the test are similarly difficult to interpret. This marked inconsistency in growth hormone levels casts some doubt on its importance as a metabolic regulator during fasting. Other studies have shown the fasting response of growth hormone in pigs to be minimal and in sheep to be absent (71).

Glucose tolerance tests. In the glucose tolerance tests before the fast, there was a prompt insulin response, the highest levels occurring within 1 minute of the end of the glucose infusion. After the fast, there was still a prompt insulin response but of a lesser magnitude. The regressions of insulin and glucose demonstrate a diminished threshold of sensitivity to glucose with an increased theoretical intercept of glucose concentration at which insulin concentration is 0 (Figure 6). However, the sensitivity of insulin release to glucose above this elevated threshold does not appear to be less in this small series of subjects. Fatty acids decreased from their high levels, but remained markedly elevated for each level of glucose or insulin concentration, suggesting a diminished sensitivity of adipose tissue α, α'triglyceride lipase to insulin [assuming this to be the rate-limiting enzyme in triglyceride lipolysis and fatty acid release (72)]. One also could explain this finding by a relative decrease in adipose tissue re-esterification rate or free fatty acid removal by peripheral tissues; these processes cannot be included or excluded by the data at hand, but appear less likely.

As expected, the glucose load was removed at a greatly diminished rate after the fast. Our data do not permit us to discern clearly between failure of glucose uptake and failure to inhibit glucose outflow into the circulation; however, rough calculations indicate that the entire fall in blood glucose between 20 and 60 minutes was due to glucosuria. This must be regarded as only a crude approximation, however, as accurate sequential urine collections were not made during this short period. In any event, the effectiveness of the released insulin on glucose metabolism was markedly decreased after the fast. Conceivably, this might be secondary to the higher levels of free fatty acid blocking the peripheral uptake of glucose (73), or continuing to augment gluconeogenesis and glucose release by the liver, or both. Unfortunately, glucose specific activity was not sequentially followed during the glucose tolerance test. Whatever the cause, however, it is evident that the rapid physiologic mechanism for recognizing and correcting hyperglycemia is ineffective after a prolonged fast.

Glucose turnover. At the end of the fast, calculated glucose turnover accounted for only one-fourth of the total calories expended, and even this low figure assumes that all the glucose not recycled was oxidized to CO_2. If one accepts the published data on cerebral glucose utilization (45, 46), this theoretical quantity is barely adequate to supply the central nervous system. Since erythrocytes (74), renal medulla (75), and probably certain other tissues such as bone marrow are also predominantly glycolytic and may account for the 20 g per m^2 per 24 hours recycled via lactate and pyruvate, it would appear that very little if any glucose is available for other tissues. This once again emphasizes the role of fatty acids and acetoacetic and β-hydroxybutyric acids as the predominant fuels in the fasting state, perhaps even for brain (see below).

These studies also point out several discrepancies in our present concepts of glucose metabolism during fasting. For one, even if all the

nitrogen in the urine originated from glucogenic amino acids, these would account for a theoretical maximum in our subjects of approximately 25 g per m^2 per day of glucose, which, added to the recycled moiety of 20.9 g per m^2 per day and the amount derived from glycerol (10 g per m^2 per day), would provide a total of 55 g per m^2 per day, far short of the 85 g per m^2 per day calculated from the isotope studies. This disagreement or factitiously high turnover is compatible with exchange of labeled glucose with carbohydrate in glycoprotein (76) or in glycogen end groups (77). The glucose combusted would then be far less than that calculated by the turnover data. Other studies, using glucose-^{14}C turnover, are accordingly suspect.

Subjects fasted for more prolonged periods of time exhibit even a further reduction in nitrogen loss to values approximating 1 g per day (78), thereby providing even less precursor for gluconeogenesis. A loss of nitrogen by routes other than urine is a possibility, and recent studies of respiratory nitrogen exchange have been suggestive (79); however, the detailed and complete balance studies performed on experimental animals at the turn of the century seemingly exclude nonurinary and nonfecal loss of nitrogen other than a very small quantity in desquamated integument (80). In addition, total body nitrogen approximates 1 kg, and survival for more than 5 or 6 months would necessitate a daily loss of less than 2 or 3 g. The urinary loss is therefore obviously representative of total loss of nitrogen from the body.

This optimal nitrogen sparing and its associated minimal glucose synthesis would be incompatible with life should the brain continue to require its 100 to 150 g of glucose (45, 46) each day. Three alternatives are possible. Gluconeogenesis from fatty acid can occur via glyoxalate in lower forms, but this pathway has not been demonstrated in mammalian systems. Another possibility is a diminution of cerebral oxygen consumption (metabolic need), and a third is utilization of nonglucose substrate. Recent studies (81) have shown only the last to be true, namely, cerebral consumption of β-hydroxybutyrate and acetoacetate as a means of sparing glucose utilization and synthesis and, *pari passu,* nitrogen loss or its equivalent, muscle mass.

In summary, glucose-^{14}C turnover data appear to be factitiously high and not in agreement with total balance data. The latter demonstrate markedly diminished glucose synthesis, which, accordingly, must be associated with markedly diminished glucose metabolism by even those tissues, such as brain, which formerly were thought to be uniquely dependent on glucose as fuel. The survival value of these progressive adaptations and the need for maximal efficiency in reactions utilizing nitrogenous material place extreme emphasis on the hormonal- and metabolite-modulated control mechanisms. Insulin, for example, may play a more significant role in regulating fasting than it does during the immediate postabsorptive state.

Summary

Levels of insulin, growth hormone, and various metabolic fuels were followed throughout a 1-week fast in six normal subjects and two patients with maturity-onset diabetes. The data from the normal individuals are compatible with the hypothesis that the glucose-insulin feedback mechanism may be the primary control process regulating the release of peripheral fuel to provide energy for metabolism during fasting.

Marked variabilities in growth hormone levels and lack of correlation of growth hormone with other parameters diminish its apparent importance in the fasting process.

Metabolic balances and glucose turnover studies were performed and demonstrate again the predominance of lipid as fuel and emphasize the diminution of glucose metabolism, which, in turn, spares nitrogen stores as gluconeogenesis decreases.

Addendum

Since submission of this manuscript Exton, Jefferson, Butcher, and Park (82) have published evidence of a consistent effect of insulin in diminishing glucose release by the isolated perfused rat liver. As has been reviewed in reference 47, others have obtained similar results both *in vivo* and *in vitro.* Our data and discussion deal only with events during prolonged fasting and, as has been emphasized, are compatible with the insulin-regulated release of peripheral fuels as the primary controlling event. During feeding, it is probable and logical that the increase in

insulin may suppress hepatic glucose output both by inhibiting release of peripheral fuel and also by a direct effect on the liver itself.

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