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Research Article

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Estimated glucose production at 5-6 wk of starvation is reduced to approximately 86 g/24 hr. Of this amount the liver contributes about one-half and the kidney the remainder. Approximately all of the lactate, pyruvate, glycerol, and amino acid carbons which are removed by liver and kidney are converted into glucose, as evidenced by substrate balances across these organs.

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Liver and Kidney Metabolism
during Prolonged Starvation

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by substrate balances across these organs.

INTRODUCTION

Both liver and kidney have the enzymatic machinery to
synthesize glucose (1, 2) and the liver is generally rec-
ognized as the major source of blood glucose after a
brief postabsorptive period. Estimations of net splanch-
nic glucose production in man after an overnight fast
vary from 122 to 420 g/day (3–7), an amount which
corresponds to approximately one-half of the basal ca-
loric turnover. Were glucose production from amino
acids to continue at this rate, all body protein would
be consumed within several weeks. That a diminution of
hepatic gluconeogenesis during prolonged starvation does

occur is evidenced, however, by a reduction in urinary
nitrogen to 3–5 g/day (8, 9). In addition, ammonia re-
places urea as the principal excreted nitrogenous prod-
uct (8), and in view of the recent observations coupling
renal ammoniagenesis with gluconeogenesis (10, 11),
an evaluation of the renal and splanchnic roles in over-
all substrate balance is warranted. Preliminary reports of
some of the data to be described have appeared (12, 13).

METHODS

Subjects. 11 obese subjects were admitted to the Clinical
Center of the Peter Bent Brigham Hospital for therapeutic
starvation (Table I). Each had previously been on various
dietary regimes without success and volunteered for the
studies described. They were informed of the nature, pur-
pose, and risks involved in both the starvation and catheteri-
zation procedures.

On admission all had normal hemograms, urinalyses, chest
and abdominal roentgenograms, electrocardiograms (except
F.N. who had left ventricular hypertrophy), thyroid hormone
levels, serum enzymes (alkaline phosphatase, lactic dehy-
drogenase, glutamic-oxalacetic transaminase), and normal
concentrations of serum protein, cholesterol, phospholipids,
triglycerides, electrolytes, urea nitrogen, and creatinine.
Daily intake during starvation consisted of one multivitamin
capsule (Unicap, Upjohn Co., Kalamazoo, Mich.), 17 mEq
of NaCl, 1500 ml of water, and, intermittently, 13 mEq of
KCl. Subjects were encouraged to participate in occupational
therapy and daily walks. Of more importance, a strong rap-
port between physician and patient was established, leading,
in most cases, to maintenance of weight reduction after
termination of the studies.

Blood and urine collections. During the prestarvation pe-
riod, an intravenous glucose tolerance test was performed
in each subject by rapid intravenous injection of glucose
(0.5 g/kg body weight) with blood drawn for analysis at
10 min intervals for 1 hr after the infusion. For the re-
mainder of the studies blood specimens were obtained at
7 a.m. before the subjects arose from bed, at 3 days pre-
starvation, on the morning fasting was initiated, 3 days af-
ter initiation of starvation, and at weekly intervals there-

TABLE I

Clinical Data

Subject	Age	Sex	Height	Weight			Loss	Final body surface	i.v. GTT*	Duration of starvation
				Initial	Final	Difference				
	yr		cm	kg	kg	kg	%	m²	kg	day
A. B.	19	M	180.3	125.2	101.8	23.4	20.5	2.25	1.2	35
R. S.	21	M	180.0	160.6	138.2	22.4	14.0	2.53	1.4	35
R. R.	28	M	177.8	178.6	159.6	19.0	10.6	2.70	0.9	35
F. F.	32	M	180.3	127.2	105.6	21.6	16.9	2.25	1.0	39
P. C.	33	M	170.8	169.0	148.7	20.3	12.0	2.49	1.4	35
F. N.	49	M	177.5	132.6	109.8	22.8	17.2	2.26	0.6	38
C. R.	16	F	167.6	104.1	88.4	15.7	15.0	1.97	2.1	35
D. D.	20	F	159.0	108.9	93.0	15.9	14.6	1.94	1.7	35
M. L.	26	F	176.0	147.3	124.2	23.1	15.7	2.37	1.2	40
S. C.	33	F	163.0	117.0	103.0	14.0	12.0	2.07	0.7	35
M. B.	43	F	173.0	123.5	98.8	24.7	20.0	2.14	1.0	41

* Intravenous glucose tolerance test (0.5 g/kg) performed before weight reduction. Values expressed as absolute per cent disappearance per min.

after. These were usually taken from either antecubital vein using a tourniquet only when necessary. Whole blood, plasma, or serum were analyzed for acetoacetate, β-hydroxybutyrate, glycerol, glucose, free fatty acids, α-amino nitrogen, insulin, and growth hormone. Urine was collected in refrigerated plastic containers, and at the end of 24 hr (7:00 a.m.) the volume was measured and aliquots taken for analysis of total nitrogen, urea, ammonia, creatinine, uric acid, acetoacetate, and β-hydroxybutyrate. The various techniques have been described (8, 14) including that of growth hormone (15). During the prestarvation period, subjects were maintained on a 2500 kcal per day diet consisting of 300 g carbohydrate, 100 g protein, and 85 g fat. All had previously been on a random diet with neither weight gain nor loss immediately before the study.

Blood flow. Effective renal blood flow (ERBF) was estimated in five subjects by clearances of sodium *para*-aminohippurate (16). Subject F.N. had a single determination after 28 days of starvation. They were encouraged to drink water freely on the night before the clearance study, and after rising and emptying the bladder at 7:00 a.m., they received 1000 ml of water by mouth during a 15–20 min period, followed by supplemental amounts to maintain urine flow greater than 5 ml/min. One antecubital vein was used for blood sampling and the indwelling needle kept patent with 0.9% sodium chloride. In the contralateral antecubital vein an indwelling needle was used for a priming dose of 0.04 ml/kg body weight of a 20% solution of sodium *para*-aminohippurate (PAH) and for a continuous infusion calculated to maintain a plasma concentration of PAH of 0.01–0.02 mg/ml. After 45 min for equilibration, three urine specimens were collected at successive 30-min intervals, and blood specimens were obtained midway between voidings. Effective renal blood flow was calculated by the formula: $ERBF = C_{PAH}/(1 - \text{hematocrit})$.

Catheterization. After five subjects had fasted 35–41 days they were given 100 mg of sodium pentobarbital and 50 mg of meperidine sulfate intramuscularly and then brought to the catheterization laboratory at 8 a.m. A polyethylene catheter was advanced percutaneously into the left brachial artery. A left median antecubital vein was exposed and a

Goodale-Lubin catheter (No. 7–8) was inserted and advanced to a loosely wedged position in the right hepatic vein. The femoral vein was catheterized percutaneously and a Goodale-Lubin catheter (No. 7–8) advanced to the right renal vein. The catheters were kept patent with 0.9% sodium chloride, and their positions were repeatedly ascertained by fluoroscopy with image intensification.

Blood samples were obtained simultaneously from the arterial, renal, and hepatic veins every 15 min for four to six periods in most cases. The samples were immediately prepared for analysis of oxygen, carbon dioxide, and metabolic substrates. Blood pressure and heart rate were monitored continuously during the procedure and did not change significantly. The patients received an isovolumetric replacement of the withdrawn blood by 5% human albumin in 0.9% sodium chloride.

Hepatic blood flow was estimated at the time of catheterization in two subjects (M.B. and F.F.) using Indocyanine green (17). After injection of 0.5 mg/kg body weight of dye, arterial and hepatic plasma concentrations were determined at 3-min intervals for 21 min at 805 mμ using a Beckman DU spectrophotometer. Plasma volume was calculated from the zero-time intercept of the arterial clearance curve. Hematocrit was measured in an arterial blood sample and corrected for trapped plasma and body hematocrit according to Chaplin, Mollison, and Vetter (18). Estimated hepatic blood flow (EHBF) was calculated from the clearance constant of dye for arterial plasma (*k*), blood volume (V), and the fractional extraction of dye in the liver (E) according to the equation: $EHBF = (kV)/E$.

Cardiac output was determined from arterial dye dilution curves after single intravenous injections of 5 mg of Indocyanine green. The area under the curve was measured by planimetry. Calibration was made by analyses of two blood samples with known dye concentrations. Statistical calculations were made according to Snedecor (19).

RESULTS

In Table II are presented the circulating levels of substrates and the concentrations of insulin and growth hor-

TABLE II

Circulating Substrate and Hormone Concentrations (Venous) in 11 Subjects

Days of Fasting...	−3	0	3	10	17	24	31	35–38
Plasma free fatty acids*	0.66 ±0.06	0.71 ±0.05	1.25 ±0.09	1.360 ±0.11	1.464 ±0.12	1.55 ±0.13	1.48 ±0.09	1.60 ±0.14
Blood β-hydroxybutyrate*	0.06 ±0.01	0.07 ±0.01	1.21 ±0.19	4.30 ±0.40	5.10 ±0.26	5.78 ±0.27	5.84 ±0.39	5.85 ±0.38
Blood acetoacetate*	0.03 ±0.01	0.03 ±0.01	0.41 ±0.06	1.00 ±0.11	1.12 ±0.11	1.27 ±0.11	1.37 ±0.13	1.34 ±0.14
Blood glycerol*	0.090 ±0.009	0.092 ±0.008	0.108 ±0.017	0.110 ±0.014	0.132 ±0.027	0.154 ±0.028	0.114 ±0.013	0.124 ±0.013
Serum α-amino nitrogen*	4.38 ±0.11	4.39 ±0.12	4.32 ±0.16	4.41 ±0.14	4.15 ±0.11	3.85 ±0.09	4.01 ±0.09	3.85 ±0.11
Blood glucose*	4.95 ±0.30	4.79 ±0.20	3.63 ±0.26	3.79 ±0.27	3.76 ±0.19	3.76 ±0.19	3.78 ±0.18	3.70 ±0.18
Serum insulin‡	45 ±6	37 ±7	20 ±5	20 ±3	17 ±4	13 ±3	17 ±3	14 ±2
Serum§ growth hormone	0.6 ±0.2	1.0 ±0.5	1.4 ±0.6	1.2 ±0.3	0.7 ±0.2	0.5 ±0.1	0.7 ±0.2	0.4 ±0.1

* Values in millimoles per liter ±SEM.
‡ Values in microunits per milliliter.
§ Values in millimicrogram per ml.

mone in the 11 subjects. There was a slight but insignificant fall in glucose and insulin concentrations between the start and the end of the dietary pretreatment (day 3 to day 0). Of note, however, is the higher level of both glucose [86.2 ±1.6 (SEM) mg/100 ml, n = 11] and insulin (37 ±7 μU/ml, n = 11) compared to an age and sex-matched, nonobese population studied in this laboratory (glucose = 76.8 ±0.8 mg/100 ml, n = 119 and insulin = 15.8 ±1.0 μU/ml, n = 69, unpublished data, courtesy of Dr. J. S. Soeldner). As shown in Fig. 1, glucose concentration reached a nadir after 3 days of fasting and stayed unchanged thereafter. The initial fall
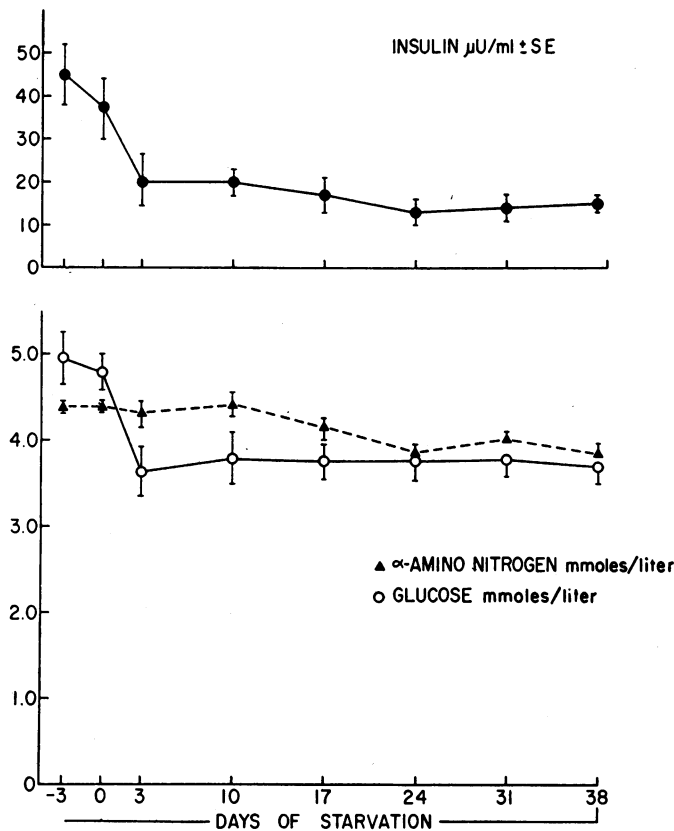

FIGURE 1 Concentration of blood glucose, serum insulin, and serum α-aminonitrogen in 11 obese subjects at various times throughout a prolonged fast.
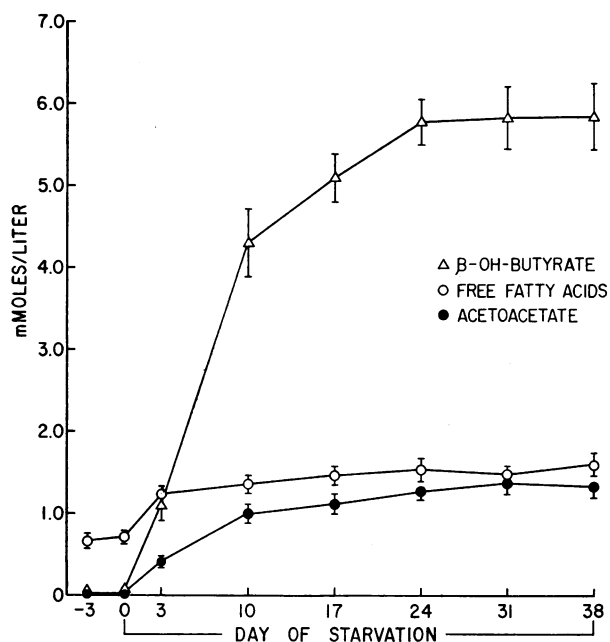
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FIGURE 2 Concentration of plasma free fatty acids and blood acetoacetate and β-hydroxybutyrate in 11 obese subjects at various times throughout a prolonged fast.

in serum immunoreactive insulin paralleled that of glucose and then decreased gradually as starvation and weight loss continued. Using the paired t test the decrease in insulin concentration between day 3 or 10 vs. day 24 is significant ($P < 0.05$ and $P < 0.01$ respectively).

Serum levels of α-amino nitrogen also fell and the decrease is significant after 17 days of fasting ($P < 0.05$). It should be stressed that this determination is a gross representation of all the amino acids and does not reflect the individual fluctuations which may be marked, a topic currently under study (20). The concentrations of β-hydroxybutyrate and acetoacetate rose slowly for the 1st 3 days (Fig. 2), and between days 3 and 24 a greater rise occurred, especially when con-

sidered in relation to the simultaneous free fatty acid concentrations. It is interesting to note that the rise in β-hydroxybutyrate and acetoacetate was associated with an increase in the ratio between the two metabolites, changing from approximately 2 at the beginning to 4.5 on the 17th day. The changes in venous glycerol levels (Table II) were not statistically significant.

In Fig. 3 is presented the urinary nitrogen excretion of a typical subject (F.N.). During the control period there were fluctuations, primarily in urea, which comprised the major component. Daily nitrogen excretion decreased progressively for the 1st 4 wk of fasting after which it remained fairly constant at about 3-6 g/day. In the five subjects who underwent catheterization studies, the mean nitrogen excretion fell to 4.7 g/day of which 42% was ammonia (Table III). Urea excretion decreased to an average of 1.55 g/day, 33% of the total.

In Table IV the ammonia excretion after 5-6 wk of starvation has been converted into mmoles/24 hr and compared to the corresponding values for excreted milliequivalents of β-hydroxybutyrate and acetoacetate. The urinary β-hydroxybutyrate: acetoacetate ratio is 8 : 1 which is greater than that of arterial blood (4.5:1). Mean total β-hydroxybutyrate and acetoacetate excretion was 114 mmoles and that of ammonia 139 mmoles, the difference probably owing to the presence of other anions as well as a possible small degradatory loss of β-hydroxybutyrate and acetoacetate in the urine in spite of storage in a refrigerator at 4°C during the 24 hr collection period.

The hemodynamic data for the five subjects who underwent catheterization are presented in Table V. For the three subjects who did not have hepatic flow determined directly and for the subject in whom no renal flow determination was made, it was assumed that 20% of the cardiac output for each represented an approximate estimation for liver and kidney blood flow (21), and further extrapolations were based on this assumption.

Successive estimates of renal blood flow were made during starvation. It is interesting to note that they failed to demonstrate any significant change (Fig. 4).

TABLE III

Grams of Urinary Nitrogen/24 hr after 5-6 wk of Starvation*

Patient	Total	Ammonia	Urea	Uric acid	Creatinine	Unidentified
A. B.	6.63	1.47	3.84	0.11	0.70	0.51
F. F.	4.53	2.70	0.88	0.09	0.67	0.19
F. F.	4.30	1.91	1.43	0.13	0.59	0.25
M. L.	2.77	1.56	0.39	0.06	0.39	0.37
M. B.	5.06	2.11	1.24	0.09	0.91	0.71
Mean ±SEM	4.66 ±0.62	1.95 ±0.22	1.55 ±0.60	0.10 ±0.01	0.65 ±0.08	0.41 ±0.09

* Values are the mean of the last 3 days of starvation.

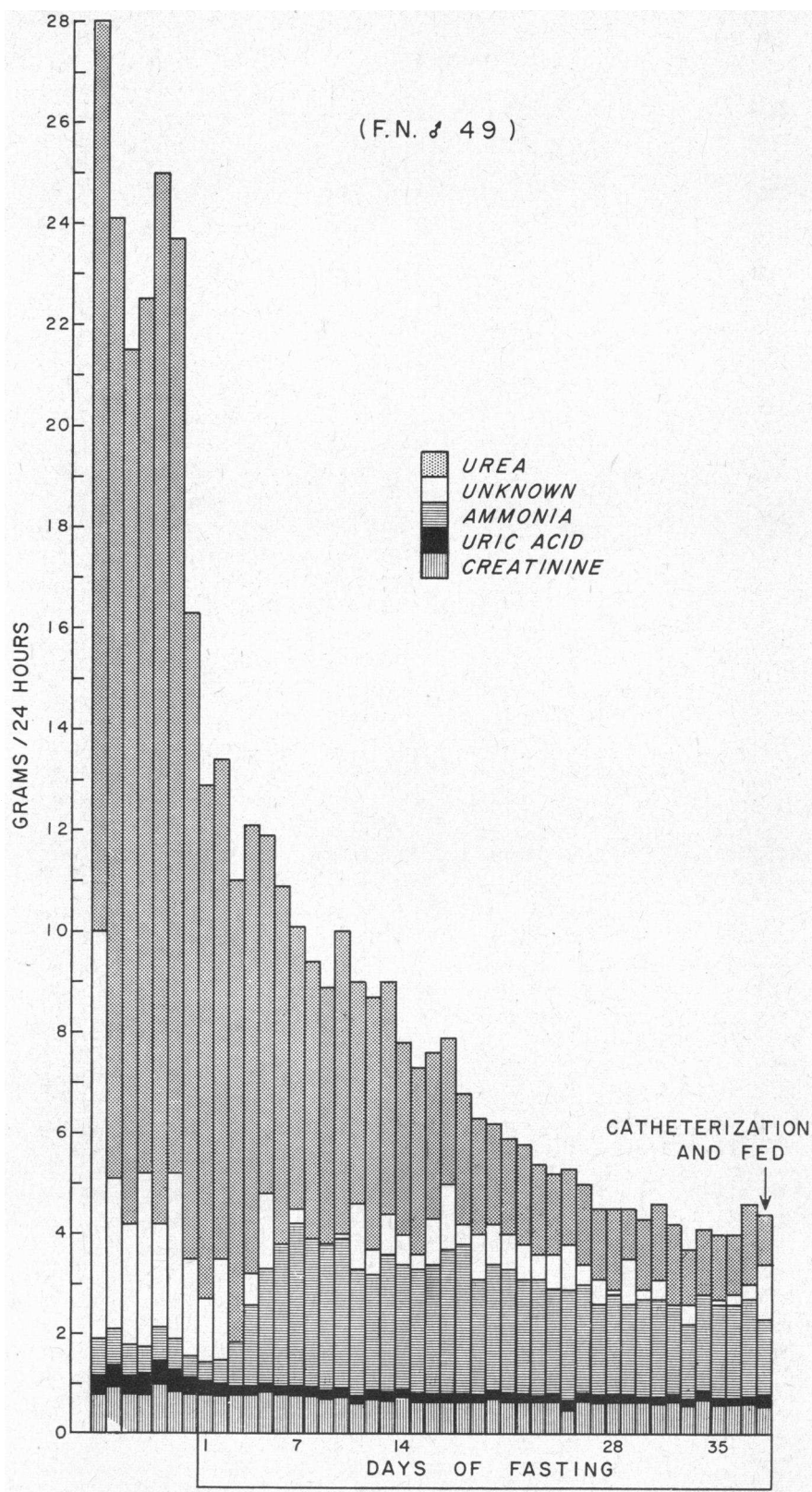*Liver and Kidney Metabolism during Prolonged Starvation* 577

FIGURE 3 Daily urinary nitrogen excretion in a male subject fasted for 5–6 wk showing baseline fluctuations during the control period and the dramatic decrease with progressive starvation with ammonia becoming the primary excreted product.

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TABLE IV

Urinary β-Hydroxybutyrate, Acetoacetate, and Ammonia Excretion (mmoles/24 hr) after 5–6 wk of Starvation

Patient	β-hydroxy-butyrate	Aceto-acetate	β-hydroxy-butyrate and aceto-acetate	Ammonia
		mmoles/24 hr		
A. B.	85	8	93	105
F. F.	138	9	147	193
F. N.	85	16	101	136
M. L.	85	—	—	111
M. B.	100	15	115	151
Mean ±SEM	99 ±10	12 ±2	114 ±12	139 ±16

* Values are the mean for the last 3 days of starvation.

The arterial levels of the various substrates after 35–41 days of starvation are presented in Table VI, as are the balances across the splanchnic and renal beds. The oxygen content and lactate and pyruvate concentrations are in good agreement with standard values obtained in nonfasting subjects, and the decreased CO_2 content is in keeping with the ketoacidosis. Of note is the low splanchnic respiratory quotient. Significant extractions of lactate, pyruvate, α-amino nitrogen, glycerol, and free fatty acids and production of β-hydroxybutyrate, acetoacetate, and glucose are observed. Although the lactate: pyruvate ratio is equal in the hepatic vein and arterial blood, the ratio of β-hydroxybutyrate: acetoacetate is lower in hepatic vein than in arterial blood, reflecting a splanchnic production of these two compounds in a ratio of 1.8: 1. Lactate, pyruvate, glycerol, and α-amino nitrogen are extracted both by the splanchnic bed and by the kidney. Free fatty acids and β-hydroxybutyrate are also extracted by the kidney, but there is a surprising and unexpected production of acetoacetate.

TABLE V

Blood Flow Rates after 5–6 wk of Starvation*

Patient	Cardiac output	Hepatic blood flow	Renal blood flow
		liters/min	
A. B.	6.1	1.22‡	1.53
F. F.	4.7	1.25	1.21
F. N.	4.2	0.84‡	1.47
M. L.	4.8	0.96‡	0.96‡
M. B.	4.5	1.13	0.88
Mean ±SEM	4.8 ±0.3	1.08 ±0.08	1.21 ±0.13

* Values in liters/min.
‡ Regional flow estimated at 20% of cardiac output (21).
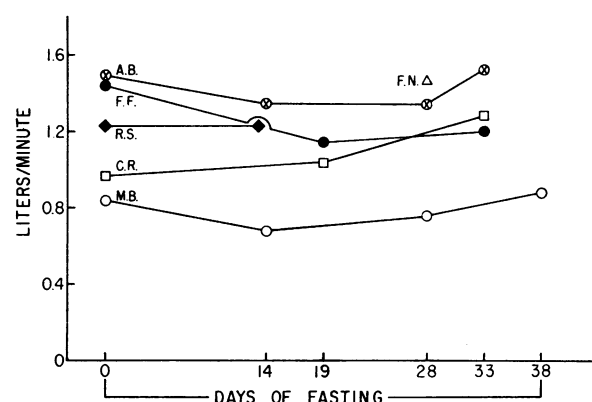

FIGURE 4 Successive determinations of effective renal plasma flow (C_{PAH}) in five subjects during starvation.

DISCUSSION

Blood insulin and substrate concentrations. Many substances including pituitary hormones, glucagon, corticosteroids, catecholamines, and prostaglandins have been shown to influence the release of free fatty acids from adipose tissue, but the possible role of insulin as the primary hormonal regulator of tissue catabolism, and thus peripheral fuel mobilization, has again been emphasized (14).

Since methods for determination of insulin in serum have become available, it has been demonstrated that obese subjects, with or without decreased glucose tolerance, have elevated insulin concentrations (22–25). Hirsch and Gallian have shown that adipose tissue cells from obese subjects exhibit hypertrophy as well as hyperplasia (26), and that insulin insensitivity is correlated with an increased lipid content in each cell (27). After the subjects lost weight and reduced their adipose cell size, insulin sensitivity and serum immunoreactive insulin levels returned to normal.

When the present obese subjects are compared to previously studied normal subjects in our laboratory (14), the obese patients exhibit the expected increased basal levels of insulin, glucose, and free fatty acids after an overnight fast. However, after 3 and 7 days of starvation substrate levels were not different between normals and obese subjects. On the other hand, insulin concentrations were 8.0 ±0.7 and 7.7 ±0.5 μU/ml in the normals compared with 20.0 ±5.0 and 20.0 ±3.0 μU/ml in the obese. Thus the persistent hyperinsulinemia observed in the starved obese subjects suggests a "relative" insulin resistance of the lipolytic mechanism of their adipose tissue during fasting. Similar results have been observed by Solomon, Ensinck, and Williams (28).

Substrate balances. Lactate, pyruvate, α-amino nitrogen, and glycerol are essentially three carbon substrates, and their sum divided by two gives an esti-

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TABLE VI
Blood, Serum, and Plasma Levels

	O₂	CO₂	RQ‡	Lactate	Pyruvate
Arterial concentrations	7.45 ±0.26	14.62 ±0.76	—	0.47 ±0.04	0.055 ±0.006
Splanchnic arterial-venous differences	2.25 ±0.07	−0.81 ±0.13	0.37 ±0.06	0.19 ±0.05	0.019 ±0.007
Renal arterial-venous differences	0.92 ±0.12	−0.61 ±0.12	0.77 ±0.21	0.043 ±0.007	0.004 ±0.001

* Values in mmoles per liter whole blood (plasma for free fatty acids and serum for alpha amino nitrogen). Each value is the mean with SEM for five subjects (A. B., F. F., F. N., M. L., and M. B.). For each subject, the value is the mean of four to six observation periods.
‡ Respiratory quotient.

mate of the amount of glucose that could be formed from these precursors if all were converted into hexose. In the splanchnic bed, 0.193 mmoles of glucose could be added to each liter of blood by these substrates, a value close to the observed 0.172 mmoles/liter. Across the kidney, the observed glucose production was 0.122 mmoles/liter and the extrapolated value from substrate differences, 0.087 mmoles/liter. These data support an optimal conversion of glucogenic substrate into glucose, presumably facilitated by the elevated levels of free fatty acids present during starvation (See Fig. 2 and Table II). Free fatty acid oxidation inhibits rate-limiting glycolytic enzymes (29), promotes increased activity of key gluconeogenic enzymes (30), and furnishes reduced pyridine nucleotides essential for glucose synthesis (31, 32).

Table VII presents balance data of free fatty acid,

TABLE VII
*Balances of Free Fatty Acids, β-hydroxybutyrate,
Acetoacetate, and Respiratory gases*

	Splanchnic*	Renal*
	mmoles/liter	
Free fatty acids	0.208	0.085
β-hydroxybutyrate	−0.335	0.279
Urine	—	−0.079
Metabolic balance	−0.335	0.191
Acetoacetate	−0.180	−0.047
Urine	—	−0.008
Metabolic balance	−0.180	−0.055
Oxygen equivalents‡	2.56	2.49
Observed oxygen balance	2.25	0.92
Carbon dioxide equivalents§	−1.26	−1.90
Observed CO₂ balance	−0.81	−0.61

* Values as mmoles per liter in whole blood.
‡ Oxygen equivalent: 23 for free fatty acid, 4.5 for β-hydroxybutyrate, and 4.0 for acetoacetate.
§ Carbon dioxide equivalent: 16 for free fatty acid, 4.0 for β-hydroxybutyrate, and 4.0 for acetoacetate.

β-hydroxybutyrate, acetoacetate, and respiratory gases. In the present study the liver extracted 19% and the kidney 8% of the arterial free fatty acid content. The latter figure, however, is probably high, the mean being elevated primarily by data from subject MB whose kidney inexplicably extracted 0.332 mmoles free fatty acids/liter of plasma. These findings and the quantities of oxygen consumed and carbon dioxide produced by the splanchnic and renal tissues are in good agreement with the data of others (4, 5, 7, 32, 33). Yet, there are stoichiometric imbalances among free fatty acids, β-hydroxybutyrate, acetoacetate, and respiratory gases. The data show greater arterio-venous differences for free fatty acids across the splanchnic and renal beds than can be accounted for by the production of β-hydroxybutyrate, acetoacetate, and carbon dioxide, and the consumption of oxygen. Possible explanations for these imbalances are repetitive errors in analysis (particularly free fatty acids such as in subject M.B.) or the lack of information pertaining to other reactions such as triglyceride synthesis (34). Of interest is the renal uptake of β-hydroxybutyrate and the production of acetoacetate, accentuated all the more when corrected for urinary loss. The data show that renal handling of these substrates is not a process of simple filtration with reabsorption, but is complicated by metabolism and interconversion.

Total balance. Using the flow data from Table V, and appreciating that these are approximations in several cases, the net exchanges for carbohydrate, β-hydroxybutyrate, and acetoacetate, and other substrates can be extrapolated to a 24 hr period of time. It must be emphasized that these calculations do not take into account diurnal changes, if they exist, changes due to activity or other inaccuracies inherent in using arterio-venous differences, and a single flow determination, particularly in a sedated subject undergoing an experimental procedure.

Certain tissues, especially the cellular blood elements, derive their energy from glycolysis without terminal

α-amino nitrogen	Glycerol	Free fatty acids	β-hydroxy butyrate	Acetoacetate	Glucose
3.61 ±0.19	0.148 ±0.018	1.86 ±0.22	6.48 ±0.65	1.42 ±0.23	4.03 ±0.36
0.14 ±0.045	0.092 ±0.016	0.346 ±0.039	−0.335 ±0.063	−0.180 ±0.059	−0.172 ±0.033
0.16 ±0.04	0.040 ±0.007	0.142 ±0.052	0.270 ±0.057	−0.047 ±0.009	−0.122 ±0.023

oxidation of glucose to carbon dioxide and water. The cyclic process of glucose breakdown in peripheral tissue with the formation of lactate and pyruvate which are transported to the liver and resynthesized into glucose has been quantitatively estimated in man from glucose-1-^{14}C turnover studies (14, 35). These values for recycling metabolites vary between 27 and 58 g/day and do not appear to be related to the nutritional status of the subjects since one study was done in fasting and the other in postabsorptive subjects. A more direct estimation of anaerobic glycolysis was obtained in our study. The combined removal of lactate and pyruvate from arterial blood by the liver and kidney was estimated to be 430 mmoles/day. These precursors could theoretically form 215 mmoles or 39 g of glucose/day, in rough agreement with the ^{14}C turnover studies previously reported (14, 35).

As discussed in a previous publication, a reduction in protein catabolism during prolonged starvation is mandatory for survival (36). Data in Fig. 3 and Table III corroborate this fact, showing the total mean urinary nitrogen excretion falling to 4.66 g/day. Urea excretion decreased the most, suggesting diminished hepatic gluconeogenesis from amino acids. In contrast, the need to titrate the urinary loss of β-hydroxybutyrate and acetoacetate with ammonium cations resulted in an increased ammonia excretion amounting to 42% of the total nitrogen. In vitro experiments using rat renal cortical tissue have coupled ammoniagenesis with gluconeogenesis (10, 11), and the substrate balance data across the kidney are further support for this relationship.

The amount of glucose derived from amino acids can be estimated from urinary nitrogen excretion or from α-amino nitrogen differences across the liver and kidney. 1 g of nitrogen is equal to 6.25 g of protein, and theoretically 4.66 g of nitrogen from catabolized protein should yield about 16 g of glucose (37). Approximately 26 g of glucose can be synthesized from the amino acids

taken up by these organs as approximated by the arterio-venous differences in our studies. These estimates of glucose production are in reasonable agreement, since some of the amino acids taken up by the liver are ketogenic rather than glucogenic, and complete conversion of amino acids to glucose appears improbable.

These obese subjects, fasted 5–6 wk. mobilize approximately 190 g of triglyceride/day, as calculated by indirect calorimetry[1] yielding 19 g of glycerol from adipose tissue which can serve as a glucose precursor. In the present study the liver and kidneys together removed approximately 213 mmoles or 19 g of glycerol from the blood per day, capable of producing 19 g of glucose, a surprising agreement. This is about one and one-half times the amount Borchgrevink and Havel reported for splanchnic and renal uptake of glycerol after an overnight fast (38).

The present study has demonstrated that there is a reduction in the total amount of glucose produced during prolonged starvation. The estimated hepatic-renal glucose production calculated from the limited flow data was 86 g/24 hr. The liver contributes approximately 55% and the kidney 45% of the total. The estimated glucose quivalent is 83 g/24 hr calculated from the measured hepatic-renal uptake of lactate, pyruvate, glycerol, and α-amino nitrogen from arterial blood. About one-half of the glucose formed, as discussed above, is derived from recycled lactate and pyruvate. The remaining glucose, derived from glycerol released from triglycerides and amino acids mobilized from proteins, can be terminally combusted to CO_2. The central nervous system, deriving the major proportion of its energy from β-hydroxybutyrate and acetoacetate (32 and 6 g/day, respectively) (8), continues to utilize the major part of this glucose, about 42 g/day (8). Recent studies by Ide, Steinke, and Cahill[2] have shown that rat brain has a

[1] A. P. Morgan. Manuscript in preparation.
[2] Ide, T., J. Steinke, and G. F. Cahill, Jr. 1968. The metabolic interactions of glucose, lactate, and β-hydroxybutyrate in rat brain. *Amer. J. Physiol.* Submitted for publication.

small but significant obligatory requirement for glucose for optimal metabolism of β-hydroxybutyrate; the human brain may behave accordingly.

The β-hydroxybutyrate and acetoacetate balances cannot be explained in light of present knowledge. Splanchnic production of β-hydroxybutyrate (54 g/day) is approximately equalled by renal extraction of this substrate (49 g/day). Yet, as shown previously (8), brain requires this fuel at the rate of approximately 32 g/day. Acetoacetate production by the splanchnic bed approximates 29 g and kidney adds to this another 8 g for a total of 37 g. β-hydroxybutyrate and acetoacetate are readily interconvertible and, if treated as a single entity, production of ketoacids is approximately equal to brain and kidney consumption plus urinary loss. But to make such a balance one must first consider that all the other tissues, particularly muscle, have virtually ceased to extract these two substrates with prolonged starvation, and preliminary studies on myocardial metabolism have suggested this to be true.[3] Secondly, a conversion of acetoacetate to β-hydroxybutyrate by one or more tissues not directly studied in these experiments must be postulated, if the approximations are indeed valid. That this may be possible is suggested by the studies of Gammeltoft (39) who showed that *in situ* perfusion of starving cat liver and hind limb for 220 min gave β-hydroxybutyrate: acetoacetate ratios of 1.7 and 10.8, respectively. Furthermore, Hagenfeldt and Wahren (40) have reported net production of β-hydroxybutyrate and/or acetoacetate by exercising skeletal muscle.

General comments. We have specifically limited the discussion to the physiological observations and extrapolations derived therefrom without expanding upon mechanisms. All tissues metabolize fat or fat-derived products with but few exceptions. The glycolytic tissues which consume glucose and produce lactate derive their energy from fat metabolism in liver and kidney, the glucose-to-lactate-to-glucose cycle serving as an energy shuttle. Protein is catabolized in the minimum amount needed to provide ammonia for excretion with urinary organic acids. The deaminated and deamidated residues are converted into glucose, and this glucose plus that amount derived from glycerol is consumed by brain as its minimal obligatory amount, the other and major proportion of brain's fuel being β-hydroxybutyrate and acetoacetate, which are, again, fat-derived products.

These quantitative observations and approximations provide little direct insight, however, into the control mechanisms, but may serve, instead, as baselines for further studies into obesity, diabetes, hormonal effects, and protein catabolic states (such as trauma or sepsis), to name a few. The fact that gluconeogenesis by liver can be so markedly attenuated, and the fact that brain

can adapt to ketoacid utilization both require a reevaluation of substrate requirements and enzyme patterns of specific cell types, particularly in man. Lastly, man and his hypertrophied central nervous system may have necessitated these unique metabolic adaptations, since to date, similar adaptations in experimental animals have not been found.

ACKNOWLEDGMENTS

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