Metabolic Studies in Total Parenteral Nutrition with Lipid in Man

COMPARISON WITH GLUCOSE

K. N. JEEJEEBHOY, G. H. ANDERSON, A. F. NAKHOODA, G. R. GREENBERG, I. SANDERSON, and E. B. MARLISS

From the Departments of Medicine and Nutrition, University of Toronto, Toronto, Ontario, Canada

ABSTRACT A study was undertaken of patients on a regimen of total parenteral nutrition comparing the nitrogen balance, energy substrates, blood amino acids, immunoreactive insulin, and immunoreactive glucagon levels during the sequential infusion of nonprotein calories as either glucose alone (glucose system) or 83% as Intralipid (Pharmacia Fine Chemicals, Montreal, Canada) and 17% glucose (lipid system). These nonprotein calories were administered with a constant background of amino acids (1 g/kg per day), vitamins, and minerals. Each system was infused for a week at a time and the order of infusion randomized. In some patients whole blood arteriovenous (A-V) levels of amino acids were measured across forearm muscle.

During the glucose system there was a significantly higher level of pyruvate, lactate, alanine, and immunoreactive insulin, consistent with glucose being the principal source of energy. In contrast, during the lipid system there was a rise in free fatty acids and ketone bodies with a fall in insulin, suggesting that lipid was now the principal source of energy. Despite these two very diverse metabolic situations the nitrogen balance with both systems was positive to a comparable degree after the establishment of equilibrium. Correspondingly, A-V differences of whole blood amino acid nitrogen showed uptake by muscle to an equivalent degree with both systems.

Clinical studies indicated that the lipid system as defined herein could be infused by peripheral vein for up to 43 days with resultant weight gain, elevation of serum proteins, and healing of fistulae. Our studies suggest that for both metabolic and clinical reasons exogenously infused lipid is a suitable source of nonprotein calories.

This work was presented in part at the Symposium on Fat Emulsions in Parenteral Nutrition, Chicago, June 1975, and at the 35th Annual Meeting of the American Diabetes Association, New York, June 1975.

Received for publication 10 June 1975 and in revised form 25 August 1975.

INTRODUCTION

Total parenteral nutrition is the accepted modality for support of malnourished patients in whom gastrointestinal disease prevents adequate oral feeding. Its major aims are protein anabolism, restoration of fluid, electrolyte, vitamin, and trace metal deficiencies, and provision of an exogenous source of nonprotein calories to meet energy requirements. Since the ultimate aims of such parenteral nutrition are the preservation and restoration of essential body tissues, all of which contain protein as an important constituent, it is desirable that the nonprotein calories infused should promote optimal nitrogen retention. The studies of Gamble (1) have led to the use of glucose as the main "protein-sparing" agent. In North America hypertonic glucose is widely employed as the only source of nonprotein calories, the approach introduced by Dudrick et al. (2, 3). Exogenously infused lipid has also been employed to provide nonprotein calories in total parenteral nutrition (4–6), and since available lipid emulsions are isotonic, they have the advantage of being suitable for peripheral infusion. Clinically, the use of lipid has been shown to promote fistula healing and weight gain in patients with inflammatory bowel disease (6) and, in preliminary studies, to promote nitrogen retention to the same extent as glucose (7, 8). However, the levels of energy substrates and hormones and detailed alterations in nitrogen balance have not been studied.

The present study was designed to provide constant nonprotein calories either as glucose or chiefly lipid in isocaloric amounts with a constant background of identical amounts of exogenous amino acid as casein hydrolysate. The two caloric sources were compared sequentially in random order in the same patients.

Such constant infusion of nutrients afforded us the opportunity to study the "fed" steady state, since nitrogen balance became stable during such infusions. To assess the metabolic responses obtained, observations were made of circulating hormones, energy substrates, and amino acids during glucose and lipid infusions. Furthermore the metabolic response of muscle to the infused amino acids was studied by measuring forearm arteriodeep venous differences of amino acids.

Finally total parenteral nutrition with lipid as a major source of nonprotein calories was given for longer periods by peripheral vein to determine whether this approach and route of administration could promote nitrogen retention and clinical improvement and whether it could be tolerated by peripheral vein without side effects.

METHODS

Patients

Patients who were referred to the gastroenterology service of the Toronto General Hospital with inability to achieve adequate nutrition via the oral route were selected for this study (Tables I and II). Those with hepatic, renal, or cardiac insufficiency, as well as those with insulin-dependent diabetes, were not included in the study. Patients with systemic infections (septicemia) were also excluded, though many had active localized inflammatory and infective processes, generally intra-abdominal. All had experienced a period of protein and caloric malnutrition before study, but this was of variable duration and severity by virtue of the time-course and nature of the diseases involved. In each instance, the nature and purposes of the study were ex-

TABLE I
Patients in Protocol I

Patient no.	Age	Sex	Height	Weight	% of ideal body wt	Serum albumin	Primary diagnosis
			cm	*kg*		*g/100 ml*	
1	55	F	150	34.0	77	2.3	Carcinoma, duodenum with obstruction.
2	59	M	178	52.8	71	3.1	Cachexia and nausea with inability to eat following open heart surgery.
3	48	F	158	45.4	94	2.5	Crohn's disease with massive resection.
4	27	F	168	48.3	83	2.7	Active Crohn's disease.
5	40	F	152	38.3	78	2.7	Postgastrectomy malabsorption.
6	20	F	163	35.2	70	2.8	Crohn's disease with pyloric obstruction.
7	23	M	178	52.0	76	2.3	Pancreatitis.
8	69	F	168	46.3	79	2.6	Postresection ileal fistula.
9	56	M	180	47.4	67	2.9	Pancreatectomy for carcinoma.
10	25	F	168	40.0	70	3.2	Active Crohn's disease.
11	40	F	158	50.4	100	3.6	Active Crohn's disease with resection.
12	22	F	163	49.0	90	2.4	Traumatic duodenal fistula.
13	35	M	173	41.8	64	2.3	Pancreatitis.
14	65	F	173	39.3	64	2.8	Carcinoma, ampulla of Vater.
15	38	F	163	38.8	70	2.4	Scleroderma with esophagitis.
16	22	M	163	47.8	82	3.5	Active Crohn's disease.
17	60	M	173	64.0	90	2.7	Pancreatitis.
18	40	F	180	51.4	77	1.8	Crohn's disease.
19	44	F	178	47.0	72	2.8	Crohn's disease with massive resection.
20	28	M	168	59.2	95	3.5	Active Crohn's disease.
21	61	M	158	55.8	91	2.8	Infarction of bowel with massive resection.
22	58	F	160	37.2	71	2.5	Crohn's disease with abdominal inflamatory mass.
23	32	F	168	52.0	90	3.1	As for patient 22.
24	25	M	178	54.9	79	3.1	As for patient 22.

TABLE II

Patients in Protocol II

Patient no.	Days*	Route‡	Age	Sex	Height	Weight	% of ideal body wt	Input§	Wt gain	Nitrogen balance	Diagnosis	Clinical result
					cm	*kg*		*kcals/kg*	*kg/wk*	*g/day*		
1	14	P	25	F	168	34.5	60	54.7	3.0		Crohn's disease with fistula.	Fistula healed.
2	43	P	35	F	160	33	64	49.7	1.27	+3.28	Crohn's disease with fistula.	Fistula closed to a pinhole.
3	39	P	34	F	168	34.3	59	45.2	1.07	+4.47	Postradiation bowel fistulae.	Fistula healed postsurgically.
4	27	C	64	F	147	40	79	31.3	1.48		Diverticulities with postoperative fistula.	Fistula healed.
5	27	P	60	M	172	57.1	86	35.7	0.90	+3.1	Postoperative bowel fistula.	Fistula healed.
6	23	P	64	M	175	69.4	95	28.6	0.31	+1.2	Postesophagectomy, anastomotic leak, diabetes mellitus.	Leak reduced to a minimum, subsequently stopped.
7	25	P	15	F	161	42.8	83	40.0	0.28	+0.80	Crohn's disease with fistula.	Fistula healed.
8	14	P	33	F	158	44	86	36.2	0 (no loss)		Crohn's disease with partial obstruction.	Operation for obstruction.
9	15	C	44	F	148	46	91	43.2	0.95		Postoperative peritonitis and ileus.	Resolution of inflammation.
10	10	C	65	F	167	48.5	84	41.2	2.14	+1.83	Postoperative fistula after irradiation of bowel.	Fistula closed. fistula.
11	11	C	69	F	158	46.8	93	40.0	1.87		Postoperative fistula.	Fistula closed to a small opening.
12	26	P	31	M	180	73	95	35.3	0.81	+0.84	Pancreatitis.	Resolved.
Mean±SEM										+2.22 ±0.54		

* Days of total parenteral nutrition using lipid system alone.

‡ P, peripheral vein; C, central vein.

§ Input of nonprotein calories.

plained to the patient and informed consent was obtained for investigative aspects of the study. Clinical aspects of total parenteral nutrition were supervised in specialized areas of the hospital by a team consisting of gastroenterologists, clinical fellows in gastroenterology, specially trained nurses, and clinical pharmacists. The latter ensured the exact formulation and provision of the infusions. All nutrients were given parenterally and only sips of water or ice were permitted by mouth.

Experimental design

Two main groups of protocols were employed.

PROTOCOL I

In 24 patients (nos. 1–24, Table I) 27 studies were done. Total parenteral nutrition was provided aseptically through a central venous catheter inserted into the superior vena cava by infraclavicular percutaneous puncture of the subclavian vein. In none of the patients was there evidence of catheter-related sepsis during the study.

All received 1 g/kg of protein as 5% casein hydrolysate with 5% dextrose (Amigen, Travenol Laboratories, Morton Grove, Ill.). They also received electrolytes (Na, K, Cl, Mg, Ca), vitamins, and trace metals (Zn, Cu, Mn, Cr, and I) in amounts indicated previously (7, 9). In addition to this basic regimen, which continued throughout the study, the patients were given either 50% dextrose (referred to as the "glucose system") or 10% Intralipid (Pharmacia Fine Chemicals, Montreal, Canada) (referred to as the "lipid system") to provide nonprotein calories amounting to 40 kcal/kg per day. The glucose system provided all nonprotein calories as glucose. The lipid system provided 83% of calories as Intralipid, which consists of soya bean triglyceride (10 g/100 ml), egg-yolk phospholipids (1.2 g/100 ml), and additional glycerol (2.5 g/100 ml) to make it isoosmolar. The FFA content was found to be negligible. The remaining 17% of nonprotein calories in the lipid system was glucose in the hydrolysate preparation. Each system was infused for 1 wk, then alternated with the other system. In three patients the studies were performed on two separate occasions (nos. 3, 4, and 12; Table I). The order of infusion was randomized: in 13 patients the lipid system was infused before the glucose system, and in 14 patients the glucose system was infused before the lipid system.

Nitrogen balance. In all patients 24-h collections of urine, stool, fistula fluid, and gastrointestinal drainage were made with appropriate preservatives. Urine collections were analyzed for creatinine to assure completeness of collection. Nitrogen was determined by a micro-Kjeldhal technique (10).

Blood sampling. Venous blood was drawn with minimal stasis from a superficial antecubital vein on days 1, 3, and 7 of the dextrose and lipid systems. The sample on day 1 of each system was drawn 2 h after the start of the system (glucose or lipid). While the antecedent nutritional

status of the patient was difficult to assess and variable, blood drawn during the second system was clearly drawn after the known and constant intake of the system (glucose or lipid) given during the preceding week. Because of randomization the effects of changing from glucose to lipid, and the reverse, could therefore be studied.

The blood samples were analyzed for glucose, lactate, pyruvate, FFA, β-hydroxybutyrate, acetoacetate, glycerol, insulin and glucagon, and amino acid levels. All samples were taken at 0900 h, with the exception of three patients whose blood was drawn at 0900, 1300, and 2100 h on the same day (day 7) to determine whether values obtained at 0900 h were representative of those prevailing throughout the day.

Biochemical methods. Blood for analysis of glucose, lactate, pyruvate, glycerol, β-hydroxybutyrate, and acetoacetate was deproteinized, immediately after its withdrawal, in an equal volume of chilled 10% (wt/vol) perchloric acid. Plasma for FFA and hormone assays was obtained from heparinized blood collected in a volume of Trasylol (FBA Pharmaceuticals, Pointe Claire, Quebec, Canada; 10,000 kIU/ml) equal to one-tenth of the volume of the blood added. The hematocrit was obtained on each sample to allow for correction of the plasma dilution so introduced. Further samples of plasma were obtained with EDTA for triglyceride and cholesterol estimations. For amino acid estimation, blood was deproteinized in cold 20% (wt/vol) trichloroacetic acid (TCA),[1] reextracted, and the TCA removed with ether as previously described (7, 9). All samples so obtained were kept on ice and centrifuged with minimal delay in the cold, and the supernates were frozen at −20°C until assay (except for triglycerides and cholesterol, for which the plasma was not frozen). Under the conditions employed, and with aliquots prepared such that a sample was thawed only once before assay, no significant decrease in concentration of these substances occurs in association with storage (11).

Glucose was measured by the glucose oxidase system employed in the Beckman glucose analyzer (Beckman Instruments, Inc., Palo Alto, Calif.). Microfluorimetric adaptations of standard enzymic methods were used, employing an Aminco Fluoromicrophotometer (American Instrument Company, Division of Travenol Laboratories, Inc., Silver Spring, Md.), for determination of lactate (12), pyruvate (13), β-hydroxybutyrate (14), acetoacetate (15), and glycerol (16). Plasma FFA were estimated by the radiochemical microtechnique of Ho (17). Repeated measurements of FFA in our samples stored for variable periods of time give comparable results, indicating that the techniques used for sampling and storage are unlikely to have caused a spurious elevation of FFA values. In addition, a positive arteriovenous (A-V) difference for FFA was observed across the forearm during the lipid system (unpublished data), suggesting that intravascular lipolysis is a physiological process rather than an artifact of sampling.

Immunoreactive insulin was determined with an anti-beef insulin antiserum (kindly furnished by Dr. Peter Wright, Minneapolis, Minn.), purified human insulin standard (25.7 μU/ng), and ^{125}I-labeled pork insulin (supplied by the Novo Research Institute, Copenhagen, Denmark) and a dextran-coated charcoal separation of free from bound hormone (18). The plasma determinations of immunoreactive glucagon were performed with antiserum 30K (obtained from Dr. R. H. Unger, Dallas, Tex.), purified pork glucagon standard, and ^{125}I-labeled pork glucagon (supplied by Novo Research Institute) and the same dextran-coated charcoal separation technique. This antiserum is considered to be relatively specific for the native hormone of pancreatic alpha-cell origin. However, it does cross-react with a substance in the globulin fraction of plasma (19), termed "interference factor" by Weir and colleagues (20) and precipitated in organic solvent extractions of plasma. Plasma levels of this material have been shown to be constant previously. For this reason a comparison of glucagon levels using paired differences in the two (glucose and lipid) systems in the same patient is a justifiable way of testing the significance of changes in relation to glucose or lipid infusion. The results of duplicate assay after ethanol extraction of the same sample in several subjects support the validity of this approach.

Statistical analysis of differences. There was considerable interindividual variation in several of the parameters, clearly related to the varied clinical disorders. Therefore, differences between values obtained during the glucose and lipid systems were tested for statistical differences by Student's *t* test, usually for paired differences (21), or the Wilcoxon rank test (22). Values are given as the mean±SEM with *n*, the number of observations, in following parentheses where necessary.

A-V difference studies. These were performed across forearm muscle; the techniques were as previously modified (23) from those originally described by Zierler (24). Briefly, a standard catheter (Argyle Medicut, 18 gauge × 5 cm, Sherwood Medical Industries, St. Louis, Mo.) was introduced retrograde into the median antecubital vein and advanced the maximum distance possible into the forearm (usually about 4 cm). A three-way stopcock allowed for either slow infusion of physiologic saline or blood sampling. A narrow sphygmomanometer cuff was placed around the wrist and the pressure elevated to above arterial level for 7 min to eliminate circulation to the hand. At the end of that period, simultaneous samples were obtained from the deep forearm vein and a contralateral artery (radial or femoral), the latter by a single puncture with a 20 gauge needle. The two studies in the same individual were performed on day 7 of each infusate system, and care was taken that precisely the same deep forearm vein in the same arm was used in these paired studies.

PROTOCOL II

In a further group of 12 patients (nos. 1–12, Table II), the 83% lipid calories infusion ("lipid system," described above) was given for longer periods (10 to 43 days) with a view to determining if such a lipid infusion was clinically effective in meeting the needs of total parenteral nutrition. Again all nutrition was given parenterally and only sips of water were permitted by mouth. In 8 out of the 12 the infusion was given exclusively by peripheral vein. This was accomplished by attaching a Y-connector to the hub of the peripheral venous cannula with the Amigen-dextrose given through one limb and the Intralipid through the second limb of the Y-connector. Both solutions ran continuously throughout the 24-h period. In the remainder suitable peripheral veins were not available because of previous intravenous therapy having caused thrombophlebitis. This regimen, in which the Amigen-dextrose mixture is diluted continuously by the Intralipid, presents the peripheral vein with an osmolality of about 467 mosmol/kg. The regimen provided the equivalent of 1 g/kg ideal body weight per

[1] *Abbreviations used in this paper:* A-V, arteriovenous; SGOT, serum glutamic oxaloacetic transaminase; TCA, trichloroacetic acid.

day of protein and 40 kcal/kg ideal body weight per day as nonprotein calories.

In these patients the following variables were observed.

(*a*) Daily monitoring for clinical side effects.

(*b*) Daily inspection of peripheral infusion—the intravenous site was changed when necessary by a trained nursing intravenous team and the number of days at each site recorded.

(*c*) Signs of subjective and objective improvement in the patient's clinical condition.

(*d*) Daily weight.

(*e*) Measurement of blood levels of electrolytes, blood urea nitrogen, plasma glucose, cholesterol, and triglycerides —three times a week.

(*f*) Measurement of hemoglobin, hematocrit, serum osmolality, serum creatinine, bilirubin, serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, calcium, phosphate, and magnesium—twice a week.

(*g*) Nitrogen balance was performed in seven patients (nos. 2, 3, 5–7, 10, and 12; Table II).

RESULTS

Protocol I

The values for all variables measured in the 0900 h blood sample on day 7 were found to be representative of those prevailing throughout the 24-h period (data not shown). The degree of change in substrate and hormone levels was independent of the order of infusion, and therefore results from each system were pooled for presentation, irrespective of whether it was in the first or second period.

Substrates. Despite differing rates of glucose administration, blood glucose levels on day 7 (Fig. 1) were comparable in the glucose and lipid systems, being (mean ±SEM) 107.4±8.79 mg/100 ml ($n = 11$) and 118.3± 5.73 mg/100 ml ($n = 10$), respectively.

On day 7 of infusion the pyruvate and lactate levels (Fig. 1) were significantly higher in patients in the glucose system compared with those in the lipid system, being 140.4±13.4 μM ($n = 11$) and 1,877±245 μM ($n = 11$), respectively, for the glucose system, compared with 85.5±12.0 μM ($n = 12$) and 869.3±87.3 μM ($n = 12$), respectively, for the lipid system ($P < 0.01$). At no sampling interval was the lactate/pyruvate ratio different in the two systems.

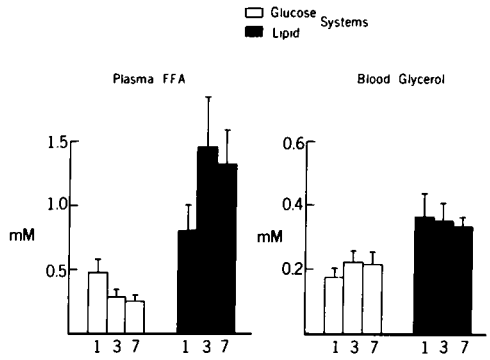

FIGURE 2 Plasma FFA and blood glycerol levels in patients during the glucose and lipid systems. The values are mean±SEM. The figures along the abscissa refer to the day of infusion of each system. The number of observations and statistical analysis appear in the text.

The FFA levels on day 7 in patients in the glucose system (Fig. 2) were about five times less than those in the lipid system, namely, 243.5±43.38 μM ($n = 10$) and 1,321.69±260.77 μM ($n = 11$), respectively ($P < 0.01$). The FFA levels in patients in the glucose system were suppressed by comparison with values from this laboratory for normal subjects in the postabsorptive state (545±37 μM [$n = 20$]), and thus, the values in the lipid system are markedly elevated from this reference point. Concurrent glycerol levels (Fig. 2) were 213.4± 35.49 μM ($n = 10$) and 330.91±58.68 μM ($n = 12$) in the glucose and lipid systems, respectively. The difference was not statistically significant.

The levels of both "ketone bodies," acetoacetate and β-hydroxybutyrate, were low (Fig. 3) during the glucose and significantly higher during the lipid system. Again,

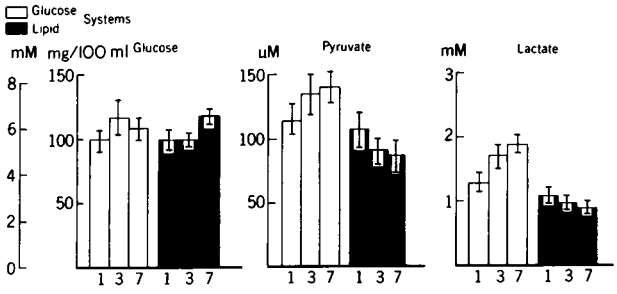

FIGURE 1 The blood glucose, pyruvate, and lactate levels in patients during the glucose and lipid systems. The values are mean±SEM. The figures along the abscissa refer to the day of infusion of each system. The number of observations and statistical analysis appear in the text.

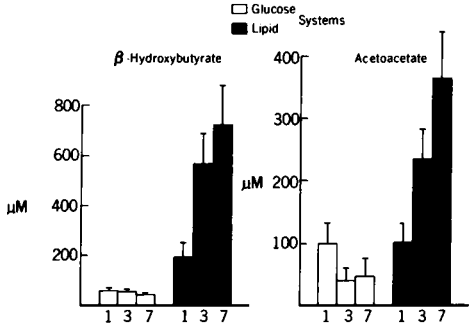

FIGURE 3 Blood β-hydroxybutyrate and acetoacetate levels in patients during the glucose and lipid systems. The values are mean±SEM. The figures along the abscissa refer to the day of infusion of each system. The number of observations and statistical analysis appear in the text.

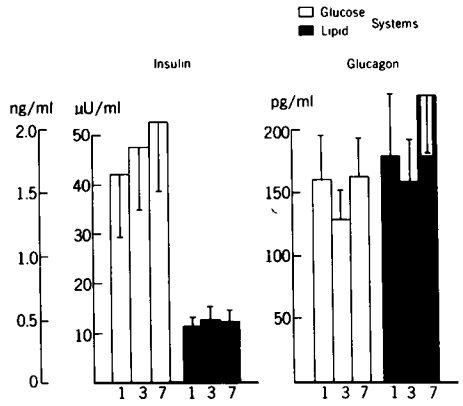

FIGURE 4 Plasma immunoreactive insulin and immunoreactive glucagon in patients during the glucose and lipid systems. The values are mean±SEM. The number of observations and statistical analysis appear in the text.

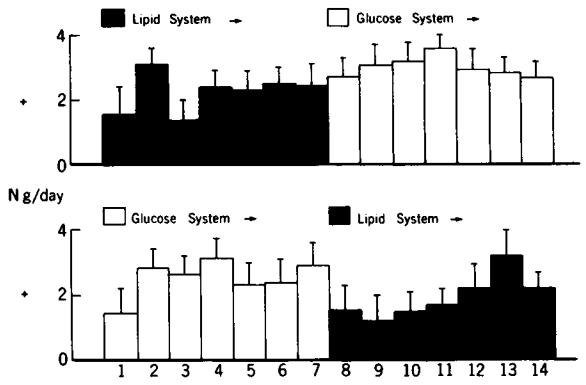

FIGURE 5 Nitrogen balance during the glucose and lipid systems. The values are mean±SEM. The figures along the abscissa refer to the day of infusion of each system. The upper half of the figure gives the results for 13 patients infused with the lipid system followed by the glucose system. The lower half shows the results for 14 patients infused in the reverse order.

on day 7, the acetoacetate and β-hydroxybutyrate levels were 48.91±22.52 μM ($n = 12$) and 42.63±7.39 μM ($n = 11$) in the glucose system compared with 371.16±64.66 μM ($n = 12$) and 726.25±138.31 μM ($n = 12$) in the lipid system ($P < 0.05$), respectively. Normal postabsorptive values in this laboratory are 332±88 μM for β-hydroxybutyrate and 110±21 μM for acetoacetate ($n = 26$).

Hormones. Fig. 4 shows mean insulin levels to be significantly higher during the glucose system on all days in which they were measured (days 1, 3, and 7). The mean day 7 insulin levels were 2.07±0.73 ng/ml (53.2±1.9 μU/ml) ($n = 11$) in the glucose system and 0.50±0.08 ng/ml (12.8±2.1 μU/ml) ($n = 11$) in the lipid system ($P < 0.05$ on testing of the means). Glucagon levels were significantly higher only when paired differences on days 3 and 7 in the lipid system were tested by the Wilcoxon ranking method (22). The glucagon levels were 162.0±31.2 pg/ml ($n = 9$) and 227.1±46.7 pg/ml ($n = 10$) on day 7 of the glucose and lipid systems, respectively.

Nitrogen balance. The results are set out in Fig. 5 and Table III. The net nitrogen balance, taken over the whole period, was positive in every patient irrespective of the system, although isolated periods of negative balance did occur. The nitrogen balance over the 7-day period was significantly higher with glucose compared with lipid (Table III). However, when examining the time-course during the 7-day period it is evident that the greater retention of nitrogen with the glucose system was only seen during the first 4 days of infusion (Table III). No significant difference was noted when the last three days (days 5-7) of each system were compared (Table III). These changes indicated that this gain of nitrogen with the glucose system was a temporary phenomenon, observed only during the first 4 days of infusion. This observation was confirmed in three patients in whom the alternation was repeated twice during the same study. In these patients, nitrogen retention was comparable during the lipid (4.44±0.19 g/24 h) and glucose (3.56±0.12 g/24 h) (P = NS) on days 5-7 (Table IV).

Amino acid levels. Whole blood individual amino acid concentrations were measured in 16 patients. In eight the order of infusion was glucose followed by lipid and in the other eight it was lipid followed by

TABLE III

Nitrogen Balance (Positive, grams/day) in Glucose and Lipid System (Mean±SEM)

Period of balance	Lipid followed by glucose				Glucose followed by lipid			
	Lipid	Glucose	n	P*	Glucose	Lipid	n	P*
	g/day				g/day			
Days 1–7	2.27±0.24	2.99±0.20	86	<0.005	2.49±0.21	1.89±0.21	95	<0.001
Days 1–4	2.15±0.34	3.14±0.28	50	<0.005	2.55±0.28	1.46±0.28	55	<0.001
Days 5–7	2.41±0.34	2.78±0.30	36	NS	2.40±0.33	2.52±0.31	40	NS

* P value is that for significance of difference between means by paired t test.

TABLE IV
Nitrogen Balance of Days 5–7

Patient no. (Table I)	System of infusion	Positive nitrogen balance*
		g/day
16	Glucose	2.8
	Lipid	4.0
	Glucose	3.25
17	Lipid	5.9
	Glucose	4.2
	Lipid	3.75
	Glucose	3.65
22	Lipid	4.59
	Glucose	3.91
	Lipid	3.97

* t test showed no significant difference between glucose and lipid systems ($0.1 > P > 0.05$).

glucose. During the glucose system, there was a significant elevation in levels of glutamic acid, glutamine, glycine, alanine, valine, leucine, tyrosine, phenylalanine, methionine, lysine, and histidine (Fig. 6). A corresponding depression occurred during the lipid system. Both changes were independent of the order of infusion. These differences in concentrations were significantly different on day 7 of infusion in each of the two systems. The concentrations of taurine, aspartic acid, serine, arginine, and isoleucine were not significantly different in the two regimens. The most striking rise seen was that in the alanine levels during the infusion of glucose. Of particular note, however, is the increase in levels of the branched-chain amino acids (valine and leucine) during the glucose system, despite the higher insulin levels reported above.

A-V difference of amino acid nitrogen. With both systems there was an uptake of amino acid nitrogen by muscle. For five patients whole blood A-V differences were $+203.0 \pm 60.2$ μM and $+353.9 \pm 90.3$ μM of amino acid nitrogen in the glucose and lipid systems, respectively. Statistically these values did not differ from one another.

Plasma triglyceride and cholesterol. In 11 patients preinfusion triglyceride and cholesterol concentrations were 111.2 ± 17.8 and 116.6 ± 16.0 mg/100 ml in the glucose and lipid systems, respectively. There was a significant increase in the values for both substances after a week of either infusion (for triglyceride, $P < 0.005$ and $P < 0.01$ in glucose and lipid systems, respectively, and similarly for cholesterol, $P < 0.02$ and $P < 0.001$). For 17 patients triglyceride means were 210.2 ± 24.2 and 193.8 ± 21.8 mg/100 ml on day 7 of the glucose and lipid infusions, respectively. These means were not statistically different ($P > 0.10$). Concurrent cholesterol means were 193.8 ± 21.8 and 360.8 ± 30.7 mg/100 ml and the difference for the two systems was significant ($P < 0.001$).

Protocol II

Reaction at site of infusion. The infusion could be delivered through each site of venipuncture for 2-5 days

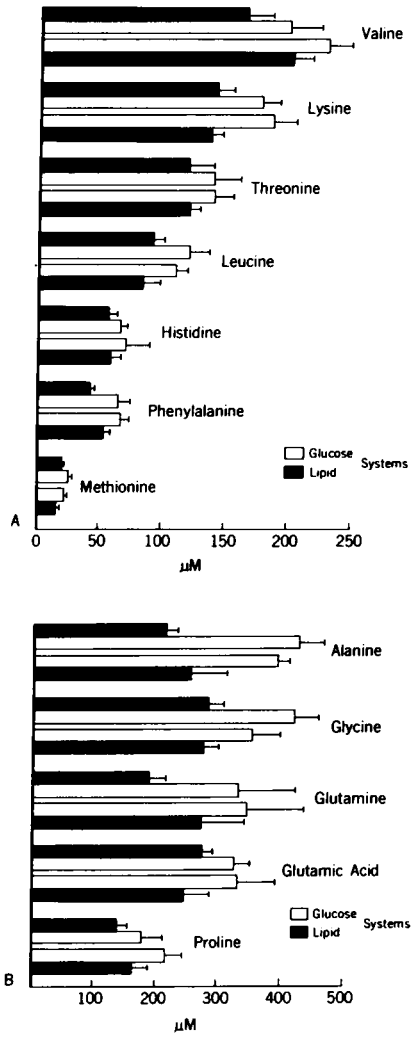

FIGURE 6 Whole blood amino acid levels on day 7 of the glucose and lipid systems, given for only those amino acids showing a significant difference between the glucose and lipid systems. The values are mean±SEM. The upper half of the diagram for each amino acid, comprising one black and one clear histogram, represents the results in eight patients infused with lipid followed by glucose. The lower half, comprising one clear and one black histogram, represents the results in eight patients infused with glucose followed by lipid. In diagram A the results of essential amino acids are set out and in diagram B those of the nonessential amino acids.

TABLE V

Serum Albumin and Liver Function Tests

Patient no. (as in Table II)	Serum albumin		Bilirubin		SGOT		Alkaline phosphatase	
	B*	A‡	B*	A‡	B*	A‡	B*	A‡
1	3.1	4.6	0.3	0.5	40	66	20	50
2	3.0	4.1	0.2	0.6	26	36	25	50
3	2.2	3.7	0.3	—	20	31	50	50
4	3.9	4.1	0.3	—	23	53	30	165
5	3.6	3.5	0.2	0.2	36	36	60	40
6	2.2	3.9	0.7	0.7	23	53	65	165
7	2.5	2.6	1.7	0.9	33	48	190	135
8	2.7	3.1	0.4	0.2	29	40	55	45
9	2.4	2.7	2.2	0.8	35	40	50	65
10	2.4	3.8	1.1	1.0	18	39	60	35
11	2.6	2.6	0.6	0.5	38	47	120	80
12	3.9	3.9	7.5	1.9	239	69	110	180

* Before lipid infusion.

‡ At end of lipid infusion.

(maximum 10 days) without phlebitis or discomfort. The patients did not experience any more discomfort than with standard 5% dextrose infusions unless the Amigen 5%-dextrose 5% solution was infused without lipid. The mixing of the two through a Y-connector was necessary to avoid discomfort.

Weight gain. This occurred in all but one patient (Table II) and was significantly correlated with the calories infused ($P < 0.05$) fitting a linear regression equation where the weight gain in kilograms per week $= -1.6 + 0.0693 \times$ kilocalories infused daily per kilogram of desirable body weight. This relationship gave an average weight gain of 0.36 kg/day when total caloric input was 60 kcals/kg per day.

Laboratory data. Blood urea nitrogen, serum electrolytes, Ca, Mg, and PO_4 levels were maintained within normal limits. The one patient with brittle diabetes (no. 6) had previously received total parenteral nutrition with hypertonic glucose and was twice recorded as being hyperosmolar due to difficulty in controlling glycemia, even with large amounts of insulin. Using the lipid system, we obtained healing of fistula and weight gain without requiring exogenous insulin.

Plasma triglyceride and cholesterol levels. After lipid administration in the 12 patients, triglyceride levels were only moderately elevated, being 127.1±11.6 and 198.7± 22.8 mg/100 ml before and after infusion ($P < 0.005$), respectively. Corresponding cholesterol levels showed consistent elevation, being 133.5±13.7 before and 422.6± 47.7 mg/100 ml after infusion. These results are similar to those seen with protocol I. In studies published elsewhere (25) it has been shown that these changes are temporary and return to normal within 2 wk of discontinuing infusion.

The changes in liver function tests were not consistent (Table V). One patient with a high SGOT level (no. 12) initially showed a fall in level during infusion while some others showed a mild rise in SGOT to below 60 IU. Alkaline phosphatase fell in two patients with initial high levels (nos. 7 and 11) and rose in three others (nos. 4, 6, and 12). Serum bilirubin level fell in three patients (nos. 7, 9, and 12) in whom it was abnormal initially. The serum albumin rose in eight out of nine patients (nos. 1, 2, 3, and 6-10) in whom it was below 3.5 g (normal serum albumin in our laboratory is 3.5–5 g/100 ml), showing that plasma protein synthesis was improved in this system of intravenously administered nutrition.

Nitrogen balance. Nitrogen balance was measured in 7 of the 12 patients in protocol II and the average of the means for each patient (measured over the whole period of infusion) was 2.22±0.54 g/24 h, which is comparable to the values observed wtih protocol I.

DISCUSSION

The present study was designed to provide metabolic data relevant both to the clinical problems of parenteral nutrition with lipid and to the determination of the hormone-substrate milieu that was associated with nitrogen retention in this lipid system (and hence with tissue repair and protein synthesis), when compared with that found in the glucose system. Furthermore in a group of patients (protocol II) studied clinically for longer periods (10-43 days), the lipid system when infused through a peripheral vein provided total parenteral nu-

trition with beneficial clinical results comparable to those seen with the use of hypertonic glucose (2, 3). In no instance did the plasma values of lipids and lipoproteins observed preclude continuance of such therapy (25, 26). This is consistent with the excellent clinical results observed previously (4, 6, 8, 27-29). Thus, the lipid system's clinical advantages of having acceptable osmolality, permitting peripheral venous infusion, must be considered as a therapeutic feature in any comparison of lipid and glucose systems.

Metabolic studies during administration of total parental nutrition using hypertonic glucose have shown positive nitrogen balance and positive elemental balances of potassium and phosphorous, indicating anabolism of muscle (30), when glucose in amounts of 15 g/kg of ideal body weight (60 kcal/kg) and the nitrogen equivalent of 2.4 g of amino acid/kg were infused. The rationale behind hypertonic glucose administration finds its historic roots in the studies of Gamble (1), who showed that within narrow hypocaloric limits (in reference to optimal caloric needs of healthy subjects), increasing the intake of glucose increased protein sparing. However, he noted the apparent paradox of the failure of added protein to achieve better nitrogen conservation than glucose alone. Hinton et al. (31) have suggested that infusion of hypertonic parenteral glucose calories with insulin can further curtail protein loss in patients with burns. The mechanism in this instance is presumed to be related to the achievement of greater concentrations of insulin in the plasma.

On the other hand, high insulin levels may not result in protein sparing under some circumstances. A recently developed concept based on some experimental data considers lower insulin levels to be desirable for optimal nitrogen retention when total caloric needs are not provided (32, 33). The reasoning behind this concept is that any moderate elevation of insulin above basal postabsorptive levels will inhibit mobilization of FFA from adipose tissue and thereby necessitate the mobilization of protein (requiring still higher levels of insulin for inhibition) to assure that the total energy needs are met (34). While low insulin levels are necessary to induce a positive nitrogen balance when endogenous calories are used, this system, to induce positive nitrogen balance (35), appears to require 1.5–1.7 g/kg per day of amino acid, which is higher than that required by the hypercaloric system (9).

The present study shows that positive nitrogen balance can be achieved in seriously ill patients with nonprotein calories given mainly as lipid, when the supplies of amino acids (1 g/kg) and total calories are comparable to (6) or lower than (30) those used with a system providing glucose as the dominant, or entire, source of nonprotein calories. Furthermore, such nitrogen retention occurs when insulin levels are as low as those seen in systems using endogenous calories (32, 33) and almost twice the amount of amino acids (35). Although the circulating levels of energy substrates and important regulatory hormones during infusion of lipid as the chief source of nonprotein calories differed markedly from those observed during glucose infusion, the nitrogen balance and uptake of amino acids by muscle appeared comparable irrespective of the major source of nonprotein calories.

During the glucose system, increased concentrations of plasma insulin and decreased concentrations of plasma glucagon were observed together with low levels of ketone bodies and FFA, but increased levels of pyruvate, lactate, and alanine in the plasma. These findings suggest that in this system glucose is being used as the principal source of energy, even though blood glucose levels are not increased. By contrast, during the lipid system there was a rise in the plasma concentrations of FFA and ketone bodies together with a fall in those of insulin, pyruvate, lactate, and alanine. While precise metabolic evidence for the relative utilization of lipid and glucose as chief sources of energy is not available, the significant rise in values for FFA and ketone bodies in association with low insulin levels, during the infusion of lipid, may suggest that these fatty acids and ketones are being used for fuel.

Nitrogen balance and A-V difference of amino acids in relation to energy source. The nitrogen balance during these two very diverse metabolic situations is of interest. Changing the source of nonprotein calories resulted in a temporary alteration in this balance during the first 4 days of each infusion. The alteration seen was a fall in nitrogen retention when the glucose regimen was changed to the lipid one and a rise when the change of system was in the reverse order. However, on the last 3 days of each infusion the nitrogen balance with the two systems was equivalent. Similar temporary changes in nitrogen retention has also been observed by others when dietary carbohydrate was replaced by fat (36). The isocaloric replacement of dietary carbohydrate with fat in that study resulted in a temporary increase in nitrogen excretion followed by a decrease to equilibrium levels. Consistent with equivalent nitrogen retention during our two systems, there was comparable uptake of amino acid nitrogen across forearm muscle, suggesting that lipid may promote anabolism of muscle to the same extent as glucose. It is clear, therefore, that any data comparing nitrogen balance in a cross-over study such as ours must take into account a period of equilibration. In this study such a time period appears to cover 4 days. Once this has been achieved, nitrogen balance becomes equivalent in the two systems. That comparable nitrogen balance can be maintained in the

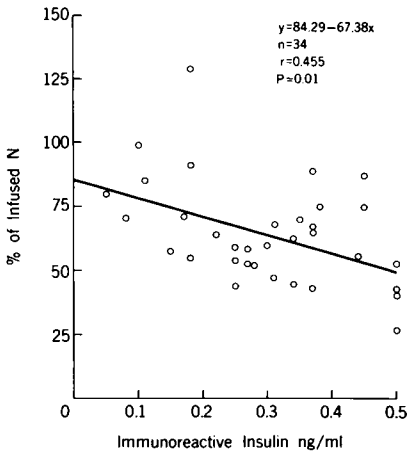

FIGURE 7 Correlation between immunoreactive insulin shown in the abscissa and total nitrogen excretion (as percentage of infused nitrogen) shown in the ordinate. Values above 100% represent negative nitrogen balance and those below 100% positive nitrogen balance. Only patients in the lipid system with immunoreactive insulin levels below 0.5 ng/ml (12.8 μU/ml) are plotted.

lipid system for longer periods of time was also demonstrated (protocol II).

Insulin concentration and nitrogen retention. Although the overall nitrogen balances observed with the two systems are equivalent with very different mean levels of insulin, there is a significant negative correlation observed in the lipid system between nitrogen excretion and insulin levels, but only at the lower levels of insulin.

In Fig. 7 nitrogen excretion is shown as a percent of the amount administered, and the data included those for all days of the lipid system for which paired values are available. In the lipid system lower insulin values were observed and a significant negative correlation ($P < 0.01$) was found on comparing the nitrogen excreted with levels of insulin when these were modest, i.e., 0.1–0.5 ng/ml (2.6–12.8 μU/ml). However, in the glucose system the value of insulin exceeded 0.5 ng/ml and did not correlate with nitrogen retention. These results do suggest that a lipid system is worthy of a controlled trial in patients known to be, or suspected of being, incapable of generating or sustaining elevated insulin levels (as illustrated in one patient—no. 6, Table II). Among such patients are diabetics and those with injury and burns (37) who may require total parenteral nutrition.

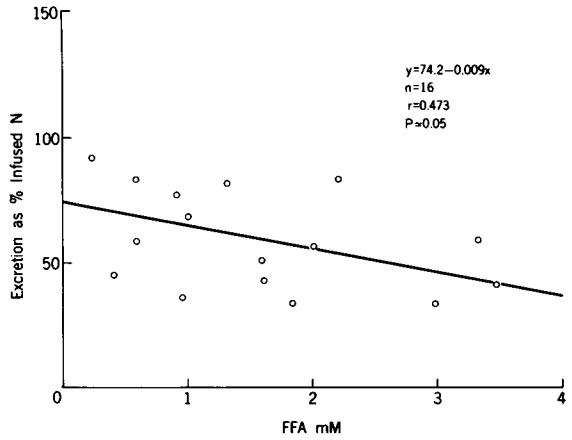

FIGURE 8 Correlation between plasma FFA levels shown in the abscissa and excretion of nitrogen (as percentage of infused nitrogen) shown in the ordinate. The values are plotted only for those patients in the lipid system who had immunoreactive insulin levels at or about 0.5 ng/ml (12.8 μU/ml).

Effects of substrate on nitrogen retention. In patients in the lipid system, with insulin levels at or above 0.5 ng/ml (12.8 μU/ml), there was a significant negative correlation between nitrogen excretion as a percentage of the amount infused with plasma FFA levels (Fig. 8). Such a correlation did not exist for values of immunoreactive insulin below 0.5 ng/ml. Considering only those patients in the lipid system who had plasma insulin levels at or exceeding the apparently critical value of 0.5 ng/ml, the negative correlation between FFA levels and nitrogen retention indicates that when this critical hormone requirement has been met, greater availability of lipid substrate improved nitrogen balance, showing a protein-sparing effect of lipid (once this certain low concentration of insulin has been achieved).

Amino acid levels in relation to energy source. The elevation in amino acids during glucose infusion (and depression during lipid infusion) is of interest and shows that under these conditions of continuous amino acid and calorie input (in excess of basal requirements) the changes in amino acid levels are in contrast with those found in the postabsorptive state following oral glucose intake. The reasons for these changes are likely to be complex. However, the increase in blood amino acid levels during the infusion of the glucose must indicate an increase in the input of amino acids or a decrease in their removal from the circulation. Increase in input cannot be from exogenous sources for the patient received a constant infusion. It is unlikely to be from endogenous sources, since an increase in amino acids from this source would involve increased protein catabolism—not likely with an equivalent (or even a temporary improvement in) nitrogen retention being observed at the same time.

Furthermore, if the rise in blood amino acids during the glucose system were due to increased release from endogenous sources, then a greater output of amino acid from muscle would be expected during the glucose system (increased negative A-V difference) in an A-V dif-

ference study. It is clear that the observed A-V difference of blood amino acid nitrogen is not consistent with an increased output from muscle during the glucose system for the A-V difference was positive with both systems.

Another possible cause of increased amino acids during the glucose system is a reduction in net hepatic catabolism of amino acids. This hypothesis is supported by the results of Ruderman and Herrera (38), who showed that amino acid catabolism and urea production were diminished in the isolated perfused liver by increasing glucose in the perfusing medium. Some increase in catabolism of amino acids would be related to the need for obligatory gluconeogenesis during lipid infusion. With the lipid system the total amount of glucose or glucogenic substrate infused, as 5% glucose and glycerol in Intralipid, would not meet the requirements (180 g of glucose per day) for the metabolism of nerve and other glycolytic tissues in the unadapted man (39), and hence, infused amino acids would be used for gluconeogenesis, contributing to the fall in levels observed with the lipid system.

Clinical tolerance and triglyceride and cholesterol levels during infusion of Intralipid. While the above results show that exogenous lipid spares protein, it is necessary to determine if this desirable metabolic feature is associated with clinical tolerance and lack of toxicity. Clinically, Intralipid has been infused without significant reactions in many centers (4, 5, 8, 27–29) and in none of our patients did the degree of ketosis result in clinically significant acidosis. The serum triglyceride concentrations were seen to increase significantly over preinfusion values but to much the same degree in both glucose and lipid systems (protocol I). More prolonged infusion of Intralipid (protocol II) did not further increase triglyceride levels. Plasma concentrations of cholesterol also increased significantly above preinfusion values with both systems but to a greater extent in the lipid system (protocol I). Again, although more prolonged infusion of Intralipid (protocol II) did cause a slight further increase, this last was not statistically significant ($P > 0.10$). In any case, the increase in plasma cholesterol was temporary (25), regressing within 2 wk of discontinuing infusion, and was not associated with any untoward side effects. On the other side of the picture, lipid infusions have been found to be necessary to avoid essential fatty acid deficiency (26, 40, 41) and development of fatty liver (26, 42, 43), a recognized complication of the glucose system. Furthermore in patients receiving intravenous feeding at home (now observed for up to 5 yr) (26, 42, 43) no untoward reaction to Intralipid has been noted with daily infusions given over several years.

In conclusion, since lipid calories induce weight gain, nitrogen retention, and amino acid uptake by muscle, comparable with those seen with glucose in seriously sick patients, the finding that nitrogen retention during lipid infusion occurs with lower insulin levels would suggest that lipid as a source of nonprotein calories should be studied further in septic and burned patients who exhibit a catabolic milieu where insulin levels are low and/or insulin resistance is present.

ACKNOWLEDGMENTS

We should like to thank for their cheerful cooperation Mr. G. Tasallas and Miss C. K. Ng of the Department of Pharmacy for formulation of the solutions used in these studies, as well as Mrs. M. Close, Miss N. Perry, and the nursing staff of the Gastrointestinal Unit, Toronto General Hospital. We are grateful for the loyal technical assistance of Regina Kurian, David Wong, Rina Thumm, Thelma Peregrino-Solomon, Peter Wei, Harry Tang, and Joan Jeejeebhoy and should like to acknowledge sincerely the help of Dr. A. Bruce-Robertson in the preparation of this manuscript and of Mrs. Janet Chrupala in its expert typing.

These studies were undertaken with the aid of Ontario Provincial Health Grant no. PR.228 and an Equipment Grant from the Toronto General Hospital Foundation.

REFERENCES

1. Gamble, J. L. 1951. Companionship of water and electrolytes in the organization of body fluids. Lane medical lecture. Stanford University Press, Stanford, Calif. **5**: 71.
2. Dudrick, S. J., D. W. Wilmore, H. M. Vars, and J. E. Rhoads. 1968. Long-term total parenteral nutrition with growth, development, and positive nitrogen balance. *Surgery (St. Louis).* **64**: 134–142.
3. Dudrick, S. J., and R. L. Ruberg. 1971. Principles and practice of parenteral nutrition. *Gastroenterology.* **61**: 901–910.
4. Hallberg, D., I. Holm, A. L. Obel, O. Schuberth, and A. Wretlind. 1967. Fat emulsions for complete intravenous nutrition. *Postgrad. Med. J.* **43**: 307–316.
5. Fekl, W. 1969. Some principles of modern parenteral nutrition. *Scand. J. Gastroenterol.* **4** *Suppl. 3*: 17–34.
6. Zohrab, W. J., J. D. McHattie, and K. N. Jeejeebhoy. 1973. Total parenteral alimentation, with liquid. *Gastroenterology.* **64**: 583–592.
7. Anderson, G. H., D. G. Patel, and K. N. Jeejeebhoy. 1974. Design and evaluation by nitrogen balance and blood aminograms of an amino acid mixture for total parenteral nutrition of adults with gastrointestinal disease. *J. Clin. Invest.* **53**: 904–912.
8. Jeejeebhoy, K. N., G. H. Anderson, I. Sanderson, and M. H. Bryan. 1974. Total parenteral nutrition: nutrient needs and technical tips. *Mod. Med. Can.* **29**: 832–841, 944–947.
9. Patel, D., G. H. Anderson, and K. N. Jeejeebhoy. 1973. Amino acid adequacy of parenteral casein hydrolysate and oral cottage cheese in patients with gastrointestinal disease as measured by nitrogen balance and blood aminogram. *Gastroenterology.* **65**: 427–437.
10. Association of Official Agriculture Chemists. 1965. Official Methods of Analysis. A.O.A.C., Washington, D. C. 19th edition. 16.

11. Girard, J. R., G. S. Cuendet, E. B. Marliss, A. Kervran, M. Rieutort, and R. Assan. 1973. Fuels, hormones, and liver metabolism at term and during the early postnatal period in the rat. *J. Clin. Invest.* **52**: 3190–3200.
12. Passonneau, J. V. 1970. Lactate. *In* Methoden der Enzymatichen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim, W. Germany. 1943.
13. Passonneau, J. V., and O. H. Lowry. 1970. Pyruvate, fluorimetric assay. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim, W. Germany. 1412.
14. Mellanby, J., and D. H. Williamson. 1970. β-Hydroxybutyrate. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim, W. Germany. 1772.
15. Mellanby, J., and D. H. Williamson. 1970. Acetoacetate. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim, W. Germany. 1776.
16. Wieland, O. 1970. Glycerol. *In* Methoden der Enzymatischen Analyse. H. U. Bergmeyer, editor. Verlag-Chemie, Weinheim, W. Germany. 1367.
17. Ho, R. J. 1970. Radiochemical assay of long-chain fatty acids using ^{63}Ni as tracer. *Anal. Biochem.* **36**: 105–113.
18. Herbert, V., K-S. Law, C. W. Gottlieb, and S. J. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* **25**: 1375–1384.
19. Valverde, I., M. L. Villanueva, I. Lozano, and J. Marco. 1974. Presence of glucagon immunoreactivity in the globulin fraction of human plasma ("big plasma glucagon"). *J. Clin. Endocrinol. Metab.* **39**: 1090–1098.
20. Weir, G. C., S. D. Knowlton, and D. B. Martin. 1975. High molecular weight glucagon-like immunoreactivity in plasma. *J. Clin. Endocrinol. Metab.* **40**: 296–302.
21. Hewlett-Packard 9810A Calculator, Model 10 Statistics Block Operation Manual. July 1971. Loveland, Colo. 5–1.
22. Wilcoxon, F. 1947. Probability tables for individual comparisons by ranking methods. *Biometrics.* **3**: 119–122.
23. Marliss, E. B., T. T. Aoki, T. Pozefsky, A. S. Most, and G. F. Cahill, Jr. 1971. Muscle and splanchnic glutamine and glutamate metabolism in postoperative and starved man. *J. Clin. Invest.* **50**: 814–817.
24. Zierler, K. L. 1961. Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. *J. Clin. Invest.* **40**: 2111–2125.
25. Jeejeebhoy, K. N., G. H. Anderson,, I. Sanderson, and M. H. Bryan. 1974. Total parenteral nutrition. *In* Tenth Symposium on Advanced Medicine. J. G. G. Ledingham, editor. Pitman Medical Publishing Co. Ltd., London. 132.
26. Jeejeebhoy, K. N., E. B. Marliss, G. H. Anderson, G. R. Greenberg, A. Kuksis, and C. Breckenridge. 1976. Lipid in parenteral nutrition. *In* Proceedings of the American Medical Association Symposium on Fat Emulsions in Parenteral Nutrition. American Medical Association, Chicago. In press.
27. Lawson, L. J. 1965. Parenteral nutrition in surgery. *Br. J. Surg.* **52**: 795–800.
28. Hadfield, J. I. H. 1966. High calorie intravenous feeding in surgical patients. *Clin. Med.* **73**: 25–30.
29. Yeo, M. T., A. B. Gazzaniga, R. H. Bartlett, J. B. Shobe, and R. N. Irvine. 1973. Total intravenous nutrition. Experience with fat emulsions and hypertonic glucose. *Arch. Surg.* **106**: 792–796.
30. Rudman, D., W. J. Millikan, T. J. Richardson, T. J. Bixler, II, W. J. Stackhouse, and W. C. McGarrity. 1975. Elemental balances during intravenous hyperalimentation of underweight adult subjects. *J. Clin. Invest.* **55**: 94–104.
31. Hinton, P., S. P. Allison, S. Littlejohn, and J. Lloyd. 1971. Insulin and glucose to reduce catabolic response to injury in burned patients. *Lancet.* **1**: 767–769.
32. Blackburn, G. L., J-P. Flatt, G. H. A. Clowes, and T. F. O'Donnell. 1973. Peripheral intravenous feeding with isotonic amino acid solutions. *Am. J. Surg.* **125**: 447–454.
33. Blackburn, G. L., J-P. Flatt, G. H. A. Clowes, Jr., J. F. O'Donnell, Jr., and T. E. Hensle. 1973. Protein sparing therapy during periods of starvation with sepsis or trauma. *Ann. Surg.* **177**: 588–594.
34. Ryan, N. T., G. L. Blackburn, and G. H. A. Clowes, Jr. 1973. Differential tissue sensitivity to elevated endogenous insulin levels during experimental peritonitis in rats. *Metab. Clin. Exp.* **23**: 1081–1089.
35. Freeman, J. B., L. D. Stegink, and L. DenBesten. 1975. The protein sparing effects of amino acid infusion at 1 and 1.7 gm/kg body weight in surgical subjects. *Fed. Proc.* **34**: 930. (Abstr.)
36. Silwer, H. 1937. Die *N*-Ausscheidung im Harn bie Einschränkung des Kohlehydratgehaltes der Nahrung ohne wesentliche Veränderung des Energiengehaltes derselben. *Acta Med. Scand. Suppl.* **79**: 5–54.
37. Allison, S. P., K. Prowse, and M. J. Chamberlain. 1967. Failure of insulin response to glucose load during operation and after myocardial infarction. *Lancet.* **1**: 478–481.
38. Ruderman, N. B., and M. G. Herrera. 1968. Glucose regulation of hepatic gluconeogenesis. *Am. J. Physiol.* **214**: 1346–1351.
39. Cahill, G. F., Jr. 1970. Starvation in man. *N. Engl. J. Med.* **282**: 668–675.
40. Collins, F. D., A. J. Sinclair, J. P. Boyle, D. A. Coats, A. T. Maynard, and R. F. Leonard. 1971. Plasma lipids in human linoleic acid deficiency. *Nutr. Metab.* **13**: 150–167.
41. Wilmore, D. W., J. A. Moylan, G. M. Helmkamp, and B. A. Pruitt, Jr. 1973. Clinical evaluation of a 10% intravenous fat emulsion for parenteral nutrition in thermally injured patients. *Ann. Surg.* **78**: 503–513.
42. Jeejeebhoy, K. N., W. J. Zohrab, B. Langer, M. J. Phillips, A. Kuksis, and G. H. Anderson. 1973. Total parenteral nutrition at home for 23 months, without complication and with good rehabilitation. A study of technical and metabolic features. *Gastroenterology.* **65**: 811–820.
43. McGill, D. B., and K. N. Jeejeebhoy. 1974. Long-term parenteral nutrition. *Gastroenterology.* **67**: 195–197.