Amino Acid Balance across Tissues of the Forearm in Postabsorptive Man. Effects of Insulin at Two Dose Levels

THOMAS POZEFSKY, PHILIP FELIG, JORDAN D. TOBIN, J. STUART SOELDNER, and GEORGE F. CAHILL, JR.

From the Elliott P. Joslin Research Laboratory in the Department of Medicine, Harvard Medical School, and the Peter Bent Brigham Hospital, and the Diabetes Foundation, Inc., Boston, Massachusetts 02215

ABSTRACT Amino acid balance across skeletal muscle and across subcutaneous adipose tissue plus skin of the forearm has been quantified in postabsorptive man before and after insulin infusion into the brachial artery.

Skeletal muscle released significant amounts of alpha amino nitrogen after an overnight fast. Most individual amino acids were released. Alanine output was by far the greatest. The pattern of release probably reflects both the composition of muscle protein undergoing degradation and de novo synthesis of alanine by transamination. A significant correlation was observed between the extent of release of each amino acid and its ambient arterial concentration.

Elevation of forearm insulin in eight subjects from postabsorptive (12 μU/ml) to high physiologic levels (157 μU/ml) in addition to stimulating muscle glucose uptake blocked muscle alpha amino nitrogen release by 74%. Consistent declines in output were seen for leucine, isoleucine, tyrosine, phenylalanine, threonine, glycine, and α-aminobutyric acid. Alanine output was insignificantly affected. Doubling forearm insulin levels (from 10 to 20 μU/ml) in eight subjects increased muscle glucose uptake in three and blocked alpha amino nitrogen output in two although both effects were seen concurrently in only one subject. Changes in net amino acid balance after insulin could be accounted for by increased transport of amino acids into muscle cells or retardation of their exit.

It is likely that ambient arterial amino acid concentrations are established and maintained primarily by the extent of muscle amino acid release. The individual amino acids whose outputs from forearm muscle decline after forearm insulinization correspond well with those whose levels fall systematically after systemic insulinization. This suggests that declines in amino acid levels after systemic insulinization are due to inhibition of muscle release. Doubling basal insulin approaches the threshold both for blockade of muscle amino acid output and stimulation of glucose uptake, effects which appear to occur independently.

INTRODUCTION

Skeletal muscle in man, by virtue of its large mass and high protein content, is a major depot for amino acids both in free and peptide-bound form. It might be anticipated that factors altering muscle amino acid extraction from plasma or release into it would profoundly influence plasma levels.

That muscle releases amino acids postabsorptively has been inferred from the progressive rise in plasma amino acids seen in eviscerated animals (3–6). A striking reduction in this rise upon insulin administration implicated muscle as a site for the action of this hormone on amino acid metabolism. The enhanced accumulation of methionine-^{35}S in muscle protein after insulin supported this concept (7). More recent studies utilizing rat diaphragm (8–10) and perfused heart preparations (11) clearly show that insulin promotes movement of amino acids into striated muscle. Insulin also increases amino acid uptake by adipose tissue (12, 13).

To relate these animal studies to intact postabsorptive man, amino acid balance across skeletal muscle and

---

Some of these data have been presented at the 81st Meeting of the Association of American Physicians, Atlantic City, N. J., 8 May 1968, and the National Meeting of the American Federation for Clinical Research, Atlantic City, N. J., 4 May 1969 (1, 2).

Received for publication 12 June 1969 and in revised form 4 August 1969.

across subcutaneous adipose tissue and skin of the forearm has been quantified. Changes after the infusion of insulin at two dose levels, directed into the brachial artery, have also been examined. In addition to describing hormonal effects on amino acid balance across peripheral tissues under physiologic conditions, these studies provide information on how insulin may regulate the flow of amino acids to liver where they can serve as glucose precursors.

METHODS

After an overnight fast, sixteen studies were performed between 8 a.m. and 1 p.m. in 14 normal male volunteers aged 22-45 yr. A polyethylene catheter was passed several centimeters into a large antecubital vein toward the wrist. Care was taken to thread the catheter as deeply as possible assuring that the venous effluent drained deep forearm tissue, mainly muscle. A superficial forearm vein, whose course could easily be seen just beneath the skin, was also catheterized. Blood collected from it drained predominantly subcutaneous adipose tissue and skin. The brachial artery was entered in the antecubital fossa and a polyethylene catheter was threaded through the arterial needle, which pointed proximally, to extend 5 mm beyond the needle tip thereby creating a second inner lumen. Evans' blue dye (T-1824) was infused continuously through the outer lumen, and forearm blood (and plasma) flow was measured by the continuous infusion dye-dilution technique (14). Solutions were delivered to the brachial artery through a 38 inch length of polyethylene tubing from a 20 ml disposable plastic syringe. Blood samples were collected in heparinized syringes from the artery proximal to the point of dye and subsequent insulin infusion by using the inner lumen. Simultaneously, blood was drawn from the two veins. Care was taken to avoid introducing heparin into the subject. A sphygmomanometer cuff placed about the wrist was inflated above arterial pressure for 5 min before and during each blood collection and during insulin infusion to exclude the hand from study. Forearm volume was determined between the wrist cuff and humeral epicondyles by water displacement.

Three metabolic sets, each consisting of an arterial, a deep, and a superficial venous blood sample, were collected at approximately 15 min intervals during a control period. Whole blood glucose was determined in triplicate by the ferricyanide method of Hoffman (Technicon AutoAnalyzer) from portions of these samples added to oxalate-fluoride tubes (15). Plasma, separated immediately by centrifugation at 4°C, was analyzed in duplicate for alpha amino nitrogen (AAN) and free fatty acids (FFA) (16, 17). A portion was precipitated with 20% sulfosalicylic acid and the protein-free supernatant fluid stored at −20°C. Subsequently, individual amino acids were measured in a single representative arterial and deep venous set using a Beckman model 120C (Beckman Instruments, Inc., Palo Alto, Calif.) amino acid analyzer (18) (glutamine, glutamic acid, asparagine, and aspartic acid are not accurately determined by this method). Serum from nonheparinized blood was analyzed for immunoreactive insulin (IRI) by a modification (19) of the Morgan and Lazarow double antibody technique (20). Arterio-deep venous difference (A-DV) and arterio-superficial venous difference (A-SV) were calculated for each metabolite in these three sets and were averaged to quantify the net balance of glucose, FFA, and AAN across muscle (A-DV) and subcutaneous adipose tissue and skin (A-SV) under basal conditions. Positive arteriovenous differences indicate an uptake or extraction and negative differences indicate an output or release.

In eight subjects after base line measurements, insulin[1] (diluted with normal saline containing 0.25% Evans' blue dye) was infused intraarterially for 26 min at a rate of 100 μU/min per kg body weight, a dose calculated to achieve high but physiologic insulin levels within the forearm. Additional sets of arterial and venous blood samples were obtained at 26 min, just before ending the infusion, and at 45, 60, and 90 min after the insulin infusion was started. Blood flow determinations accompanied each set of blood samples. Insulin-induced changes in uptake or output of each metabolite were taken as changes in A-DV (for forearm muscle) and A-SV (for subcutaneous adipose tissue and skin) when blood flow was unchanged. In the eight remaining studies, subjects received insulin at one-tenth the above infusion rate, 10 μU/min per kg body weight for 26 min. Two of these eight studies were performed on subjects previously studied at the higher infusion rate. Protocols for the "high" and "low" dose groups were otherwise identical.

RESULTS

Basal forearm metabolism. Glucose, FFA, and AAN balances across deep and superficial tissues before infusion of insulin are given in Table I. Data for "high" and "low" dose groups are combined. Significant differences between deep and superficial systems in the arteriovenous difference of each metabolite support previous observations of anatomic compartmentalization according to tissue type in the forearm (21). Both superficial and deep tissues removed glucose from arterial blood. A-SV was slightly greater than A-DV. Muscle extracted FFA from arterial blood resulting in a positive A-DV (+0.06 mEq/liter) and consistent with the known importance of FFA as an energy source for resting muscle (22–24). Adipose tissue released FFA which was

[1] Glucagon-free crystalline zinc insulin, lot C226 6B, was kindly provided by Dr. W. R. Kirtley, Eli Lilly and Co., Indianapolis, Ind.

TABLE I

*Base Line Arteriovenous Differences for Glucose, Fatty Acids, and Alpha Amino Nitrogen in 16 Studies**

	Glucose	FFA	AAN
	mg/100 ml	*mEq/liter*	*mmoles/liter*
A-DV	3.5 ±0.41‡	+0.06 ±0.020	−0.49 ±0.078
A-SV	4.7 ±0.52	−0.21 ±0.050	−0.15 ±0.046
P	<0.005	<0.001	<0.005

* Abbreviations: A-DV = arterio-deep venous difference; A-SV = arterio-superficial venous difference; FFA = free fatty acid; AAN = alpha amino nitrogen; and *P* is probability that the difference between A-DV and A-SV might occur by chance (paired *t* test).

‡ ± SEM.

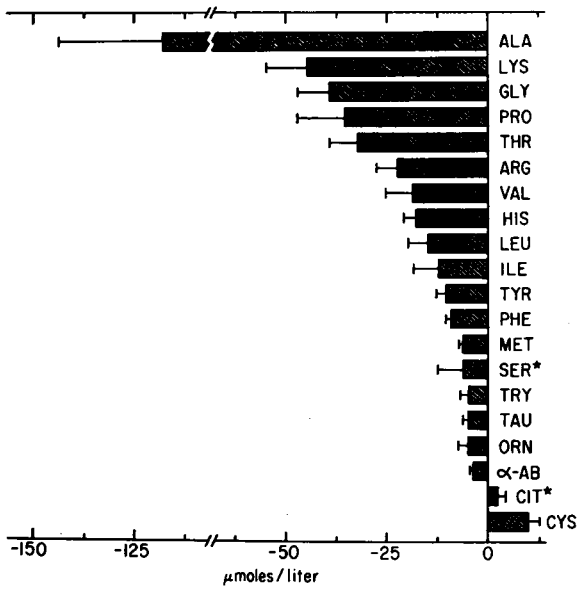

FIGURE 1 Postabsorptive balance of individual amino acids across forearm muscle (A-DV) in six subjects. One standard error of the mean A-DV for each amino acid is shown. Asterisks indicate amino acids whose mean A-DV is not significantly different from 0 (Student's t test).

reflected by a negative A-SV (−0.21 mEq/liter). Deep and superficial tissues both released amino acids measured as AAN. A-DV (−0.49 mmole/liter) was substantially greater than A-SV (−0.15 mmole/liter). While the relative contribution of skin as opposed to subcutaneous adipose tissue to AAN output of superficial tissues is not known, skin probably makes the larger contribution since its protein content is high and turnover is active (25). Adipose tissue, on the other hand, is at most 2% protein (26). Of the 22 simultaneous arterial and deep venous serum IRI determinations in these 16 studies, the arterial level was greater than the venous level in 12, equal in 5, and less than venous in 5. Resting forearm plasma flow averaged 2.2 ±0.22 (SEM) ml/min per 100 ml forearm.

In six of the eight subjects subsequently to receive the "high" dose insulin infusion, arterio-deep venous differences for 20 individual amino acids were determined in one set of base line samples (Fig. 1). Significant outputs were demonstrable for practically all with alanine making the single greatest contribution to resting amino acid release. Serine and citrulline were unusual in that neither an output nor an uptake was regularly seen. Cystine was the only amino acid consistently taken up by forearm muscle in the postabsorptive state. For each of the six subjects, large amino acid outputs were associated with high arterial levels. This relationship between amino acid A-DV and arterial level is illustrated for each subject in Fig. 2. Plasma flow need not be considered in constructing such a correlation for a particular subject since it is the same for each amino acid.

Response to maximum physiologic insulinization (*Table II*). In eight subjects given insulin directly into the brachial artery at a rate of 100 μU/min per kg body weight for 26 min, deep venous IRI rose, peaking at an average of 157 ±23.3 μU/ml. Arterial IRI proximal to the point of infusion, reflecting recirculating insulin, rose only 3.1 μU/ml (from 13.5 to 16.6 μU/ml) since exogenous insulin was diluted on entering the general circulation after leaving the forearm. Despite the slight systemic rise in insulin, there was no significant change in arterial glucose, FFA, total AAN, or individual amino acids.

Any conclusions regarding hormonally mediated alterations in metabolism based on measurements of arteriovenous difference are contingent on relative constancy of plasma flow (27). Among these subjects, as in others given insulin in a like manner (24, 28), mean plasma flow tended to rise during the infusion although

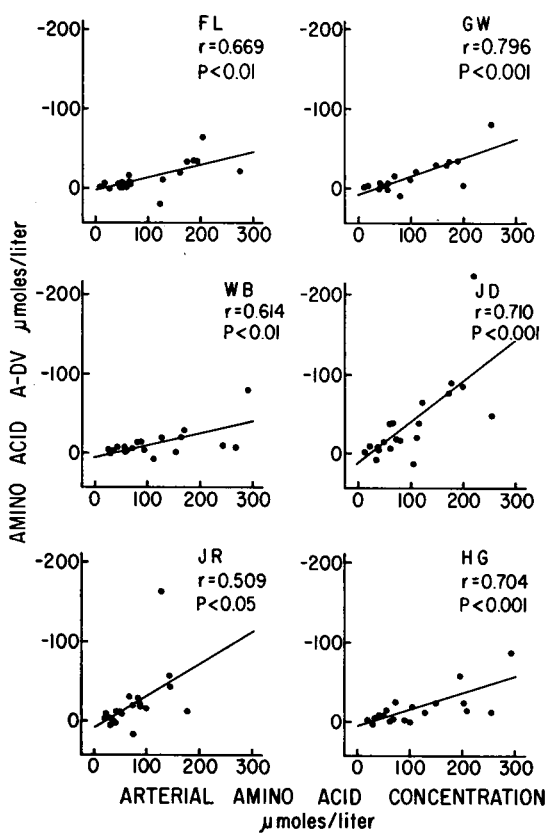

FIGURE 2 Relationship between A-DV and ambient arterial level of individual amino acids after an overnight fast in each of six subjects. Each dot represents a single amino acid. The coefficient of correlation (r), the significance of the correlation, and the calculated regression line are shown.

TABLE II

Effect of Maximum Physiologic Insulinization on Forearm Metabolism in Eight Subjects

Time after insulin started	Arterial insulin	Deep venous insulin	Plasma* flow	Glucose		FFA		AAN	
				A-DV	A-SV	A-DV	A-SV	A-DV	A-SV
min	*μU/ml*		*ml/min per 100 ml forearm*	*mg/100 ml*		*mEq/liter*		*mmoles/liter*	
0	13.5 ±0.96‡	11.9 ±0.66	2.4 ±0.48	3.5 ±0.57	4.4 ±0.56	+0.04 ±0.019	−0.14 ±0.069	−0.57 ±0.110	−0.16 ±0.088
26	16.6 ±1.39 (<0.025)§	157.0 ±23.3 (<0.001)	3.2 ±0.39	23.1 ±2.54 (<0.001)	9.0 ±2.46	+0.08 ±0.044	−0.03 ±0.027	−0.42 ±0.125 (<0.025)	−0.16 ±0.117
45	11.9 ±1.12	25.1 ±1.83 (<0.001)	2.8 ±0.25	25.1 ±3.15 (<0.001)	10.6 ±1.37 (<0.01)	+0.09 ±0.038	−0.02 ±0.020	−0.21 ±0.127 (<0.025)	−0.13 ±0.129
60	11.5 ±1.80	17.0 ±1.09 (<0.01)	2.8 ±0.35	20.2 ±2.02 (<0.001)	10.0 ±0.83 (<0.005)	+0.10 ±0.029 (<0.05)	+0.02 ±0.017 (<0.05)	−0.15 ±0.047 (<0.001)	+0.03 ±0.075
90	11.8 ±0.62	12.3 ±2.33	2.2 ±0.28	12.6 ±1.80 (<0.005)	7.7 ±1.27 (<0.01)	+0.14 ±0.028 (<0.005)	+0.04 ±0.019 (<0.025)	−0.30 ±0.072 (<0.05)	−0.21 ±0.103

* Determined in seven subjects.

‡ ±SEM.

§ Probability that number differs from 0 time by chance alone (paired *t* test), only those <0.05 indicated.

the changes were not statistically significant. Except in two subjects at the 90 min period, flow determinations in each study after the 26 min interval deviated less than 25% from the average for that entire study, assuring adequate flow constancy once the infusion had been completed.

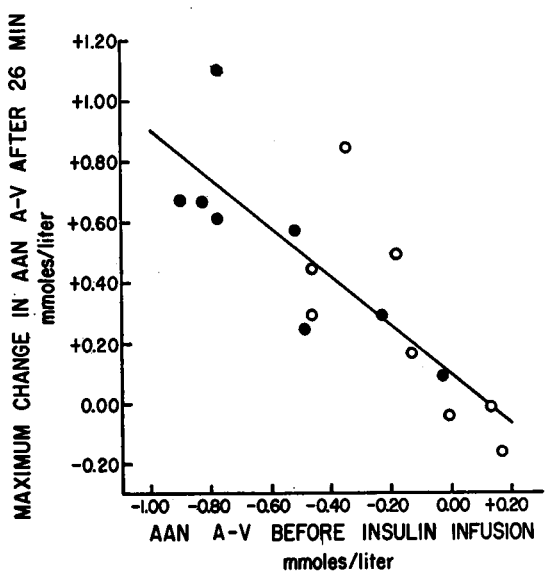

FIGURE 3 Importance of basal AAN arteriovenous difference in determining the maximum response to insulin. Closed circles represent A-DV, open circles A-SV. Because of plasma flow instability during the insulin infusion, only arteriovenous differences after 26 min were considered in quantifying maximum response. The linear regression line, calculated from all the points illustrated, is shown. The coefficient of correlation, −0.814, is significant ($P < 0.001$).

After the insulin infusion, glucose A-DV and A-SV rose to a maximum of 25.1 and 10.6 mg/100 ml, respectively. FFA release from subcutaneous adipose tissue was inhibited completely as A-SV rose from −0.14 to +0.04 mEq/liter at 90 min. The increase in A-DV for FFA from +0.04 to +0.14 mEq/liter, while possibly representing increased muscle FFA extraction, more probably reflects blockade of FFA release from lipocytes located between muscle fibers in the deep system (21, 24).

At the 60 min interval, when flow had returned essentially to base line, net AAN release from muscle had declined 74% as the A-DV, which was initally −0.57 mmole/liter, reached −0.15 mmole/liter. Insulin blocked amino acid release from muscle in all eight subjects. The magnitude of change in arteriovenous difference depended on the basal value (Fig. 3). Significant net amino acid uptake after insulin infusion was not seen; consequently, when the basal arteriovenous difference was only slightly negative, the insulin response was small. Superficial tissues appeared to behave qualitatively like muscle. When a sizable negative resting A-SV was present, it declined; and consequently, insulin-induced changes were large (Fig. 3). However, negligible resting A-SV's in three subjects were unaffected by insulin precluding demonstration of a significant blockade of release from superficial tissues for the group as a whole (Table II).

After the insulin infusion, arterio-deep venous differences for 20 individual amino acids were determined at the 60 min interval in five subjects and at the 45 min

TABLE III

Effect of Maximal Physiologic Insulinization on Individual Amino Acid Balance across Forearm Muscle in Six Subjects

Amino acid	Arterial concentration		A-DV		P*
	Before insulin	After insulin	Before insulin	After insulin	
	μmoles/liter		*μmoles/liter*		
Taurine	43 ±5.1‡	51 ±5.2	−4 ±1.3	+2 ±2.2	
Threonine	135 ±12.8	127 ±12.9	−32 ±7.1	−11 ±4.4	<0.025
Serine	107 ±5.4	107 ±6.3	−6 ±7.2	+7 ±3.7	
Proline	178 ±21.5	166 ±20.4	−36 ±11.7	−18 ±2.9	
Citrulline	32 ±1.8	32 ±2.4	+2 ±1.3	+2 ±1.0	
Glycine	179 ±8.4	175 ±11.8	−40 ±7.8	−20 ±4.6	<0.05
Alanine	231 ±25.8	212 ±26.2	−118 ±25.8	−98 ±12.0	
α-aminobutyrate	17 ±3.5	18 ±2.4	−3 ±0.3	+2 ±0.7	<0.01
Valine	238 ±16.5	230 ±16.3	−18 ±6.6	−6 ±3.0	
Cystine	86 ±5.4	93 ±10.0	+10 ±3.4	+11 ±3.4	
Methionine	21 ±1.0	20 ±1.2	−6 ±1.0	−8 ±3.1	
Isoleucine	56 ±5.7	58 ±4.0	−12 ±5.6	0 ±1.5	<0.05
Leucine	120 ±8.5	118 ±7.8	−15 ±5.0	+1 ±3.7	<0.025
Tyrosine	54 ±5.2	53 ±5.6	−10 ±2.2	−3 ±2.8	<0.05
Phenylalanine	49 ±1.5	47 ±2.0	−9 ±1.4	−6 ±2.1	<0.025
Ornithine	54 ±4.1	59 ±3.9	−4 ±2.5	+5 ±7.7	
Lysine	172 ±8.5	170 ±14.5	−44 ±10.4	−33 ±5.2	
Histidine	76 ±4.3	80 ±7.1	−17 ±2.9	−15 ±4.1	
Tryptophan	38 ±2.1	36 ±3.6	−4 ±1.8	−2 ±1.5	
Arginine	75 ±7.1	73 ±9.0	−22 ±5.4	−15 ±2.1	

* Probability that the change in A-DV after insulin is a chance occurrence (paired *t* test).

‡ ±SEM.

interval in one.[3] In Table III these values are compared to preinsulin values for the same subjects. Consistent declines in muscle release of threonine, glycine, α-aminobutyric acid, isoleucine, leucine, tyrosine, and phenylalanine were seen after insulin. Release of other amino acids decreased as well but these changes were more variable. It is noteworthy that alanine, which makes the greatest contribution to basal output, was little affected. Its release decreased approximately 17%, a change which was not significant. As indicated, arterial levels were unchanged since only forearm muscle, a small portion of total body muscle mass, was exposed to elevated insulin levels. Insulin could produce the observed changes by increasing the movement of amino acids into muscle or by decreasing their exit. Both actions are known to occur in in vitro systems (10, 29). In the present investigation changes in net amino acid balance, the algebraic sum of these two processes, have been described. The relative importance of stimulation of entry as against inhibition of exit is not determinable.

Response to a small increment in insulin. In eight subjects insulin was delivered into the brachial artery at 10 μU/min per kg body weight for 26 min, one-tenth the infusion rate used to achieve maximal physiologic insulinization. Serum IRI was measured in deep venous blood at 15 and 20 min in addition to the intervals previously used and doubled during the infusion (Table IV). Systemic (arterial) IRI did not rise significantly.

Among these subjects, the changes from control in metabolite arteriovenous differences at the 26 min interval must be interpreted conservatively because mean plasma flow increased significantly during the infusion from 1.9 to 2.8 ml/min per 100 ml forearm. After 26 min, however, plasma flow declined and did not differ from control levels. Each flow determination in all studies after the infusion was completed deviated by less than 25% from the mean for that study except in one subject at the 90 min interval.

Doubling basal insulin levels did not affect extraction of glucose by superficial tissues (A-SV). However, a slight rise in muscle glucose extraction occurred which was significant at 45 min. With regard to FFA release by superficial tissues, an antilipolytic effect was observed. Though release of adipose tissue FFA was incompletely blocked, a change of 0.17 mEq/liter (−0.27 to −0.10 mEq/liter) after a small increment in insulin equaled the change of 0.18 mEq/liter (−0.14 to +0.04 mEq/liter,

[3] For technical reasons insufficient blood was obtained at 60 min in one subject to permit measurement of individual amino acids. His peak response for AAN occurred at 45 min.

TABLE IV

Effect of a Small Increment in Insulin on Forearm Metabolism in Eight Subjects

Time after insulin started	Arterial insulin	Deep venous insulin	Plasma flow	Glucose		FFA		AAN	
				A-DV	A-SV	A-DV	A-SV	A-DV	A-SV
min	*μU/ml*		*ml/min per 100 ml forearm*	*mg/100 ml*		*mEq/liter*		*mmoles/liter*	
0	12.1 ±1.48*	9.9 ±1.19	1.9 ±0.19	3.4 ±0.58	5.0 ±0.85	+0.08 ±0.036	−0.27 ±0.069	−0.41 ±0.111	−0.14 ±0.037
15		19.9 ±1.48 (<0.001)‡							
20		18.4 ±1.12 (<0.001)							
26	13.6 ±1.78	19.0 ±1.80 (<0.005)	2.8 ±0.44 (<0.005)	4.4 ±1.25	4.3 ±0.59	+0.06 ±0.057	−0.25 ±0.065	−0.15 ±0.096 (<0.025)	−0.05 ±0.089
45	12.7 ±2.01	12.5 ±1.02 (<0.025)	2.2 ±0.45	6.4 ±1.53 (<0.025)	3.6 ±0.80	+0.12 ±0.019	−0.10 ±0.030 (<0.05)	−0.29 ±0.131	0.00 ±0.103
60	10.8 ±1.15	11.1 ±1.66	2.1 ±0.31	5.0 ±1.02	5.6 ±0.84	+0.09 ±0.035	−0.11 ±0.044 (<0.05)	−0.31 ±0.096	−0.04 ±0.078
90	7.8 ±1.93 (<0.05)	8.8 ±1.93	2.1 ±0.34	3.8 ±0.30	5.4 ±0.63	+0.12 ±0.017	−0.14 ±0.044 (<0.025)	−0.29 ±0.080	−0.15 ±0.134

* ±SEM.

‡ Probability that number differs from 0 time by chance alone (paired *t* test), only those <0.05 indicated.

TABLE V

Effect of a Small Increment in Insulin on Muscle Glucose and Amino Acid Metabolism

Minutes after start of insulin:	Glucose					AAN				
	Basal* A-DV	Change in A-DV‡				Basal A-DV	Change in A-DV			
	0	26	45	60	90	0	26	45	60	90
Subjects	*mg/100 ml*		*mg/100 ml*			*mmoles/liter*		*mmoles/liter*		
B	6.8	+3.0	+9.1	+2.9	−2.0	−0.61	+0.43	+0.95	+0.39	+0.56
H	4.7	−2.6	+0.7	−3.3	−1.2	−0.28	+0.19	+0.04	−0.12	−0.10
D	3.7		−0.1	−0.9	−1.2	−0.81	+0.49	+0.12	+0.52	+0.76
J	3.0	−1.4	+2.7	+0.1	+0.8	−0.35	−0.15	+0.10	+0.29	+0.08
O	3.4	+5.3	+7.3	+4.7	+1.7	−0.87	+0.55	0.00	−0.04	+0.16
L	3.2	+1.1	−0.2	+2.4	+1.1	−0.23		+0.08	−0.10	−0.03
Ru	1.5	−0.6	+0.6	+0.5	+1.8	−0.11	+0.01	+0.02	−0.12	−0.03
Ri	1.3	+2.8	+3.9	+5.6	+1.7	−0.04	−0.04	−0.33	−0.01	−0.43
Mean	3.4	+1.1	+3.0	+1.5	+0.3	−0.41	+0.27	+0.12	+0.10	+0.12
±SEM	0.58	0.98	1.17	0.98	0.51	0.111	0.107	0.128	0.091	0.134
High dose§	3.5	+19.6	+21.6	+16.7	+9.4	−0.57	+0.15	+0.36	+0.42	+0.23
±SEM	0.57	2.60	3.07	1.98	1.79	0.110	0.056	0.142	0.074	0.099

* Basal values are means for all determinations before insulin.

‡ Changes in A-DV are obtained by subtracting A-DV at time 0 from A-DV at time *t*. Changes at 26 min are of questionable validity because of the significant increase in flow at this time.

§ Mean values for "high" dose group are shown for comparison.

Table II) after maximum insulinization. This indicated a quantitatively similar antilipolytic activity at both insulin levels. The difference between the two groups lies mainly in the greater basal lipolysis among subjects who received the "low" dose infusion. As a group there were no changes in AAN arteriovenous difference across deep or superficial tissues when basal insulin levels were doubled except for A-DV at 26 min, a change attributable mainly to increased plasma flow.

It is apparent from an examination of individual responses to the "low" dose insulin infusion (Table V) that the mean increase in muscle extraction of glucose at 45 min was attributable to a definite response of subjects B, O, and Ri. AAN responses (excluding the 26 min interval) were evident in subjects B and D. Thus, while B responded with changes in A-DV of both glucose and amino acids, an action of insulin on movement of one metabolite without the other occurred in O, D, and Ri.

Insulin loss in preparation and delivery. In preparing the insulin infusion and delivering it into the brachial artery, one can anticipate loss of hormone from binding to glassware and plastic tubing (30). For the "low" dose studies this consideration is of special importance because of the small quantities of insulin involved. To assess these losses the stock insulin solution was assayed directly after dilution in borate buffer (pH 8.0) containing 5% bovine serum albumin. It contained 42.2 U/ml of immunoassayable insulin. In each subject on the day of study and after completion of the insulin infusion, 0.1 ml of the infusate was delivered through the same tubing into the appropriate albumin containing buffer; it was then diluted and assayed. IRI levels in the solution actually delivered to the subject could then be compared with those anticipated had there been no insulin loss in preparation and delivery. Insulin losses in the "high" dose study were negligible. An average of only 37% of the calculated dose actually reached the brachial artery in the "low" dose studies (Table VI). Disparities in the literature regarding the sensitivity of forearm tissues to small increments in insulin (31, 32) can probably be attributed to differences in insulin loss as we shall discuss later.

TABLE VI

Loss of Exogenous Insulin during Its Dilution and Infusion at 10 μU/kg per min

Subject	Calculated infusate insulin concentration	Measured infusate insulin concentration	Fraction of calculated insulin dose delivered
	μU/ml	*μU/ml*	
B	7700	2920	0.38
H	7120	2020	0.28
D	6900	2480	0.36
J	8370	4050	0.48
O	8650	3600	0.42
L	8650	2700	0.31
Ru	7540	3380	0.45
Ri	7760	1800	0.23
Mean	7840	2870	0.36
±SEM	237	275	0.030

DISCUSSION

In the present investigation amino acid balance across peripheral tissues of man has been measured directly, and alterations induced by elevating insulin within the physiologic range have been defined. After an overnight fast, skeletal muscle is in negative nitrogen balance. Subcutaneous adipose tissue and skin also release AAN, though relative to skeletal muscle the negative arteriovenous difference across superficial tissues is much smaller. The basal pattern of individual amino acids released from forearm muscle that we have observed agrees remarkably well with that described previously by London, Foley, and Webb (33). The high postabsorptive output of alanine relative to the other amino acids is striking since analysis of a wide variety of specific muscle proteins shows that alanine comprises no more than 10% of these proteins (34). Even after a 3–6 wk fast when the outputs of most amino acids including alanine have decreased, alanine release remains disproportionately high (35). Alanine is probably synthesized de novo in substantial amounts by transamination of pyruvate. This process, as well as the composition of muscle protein undergoing breakdown, contributes to the pattern of release.

By using the values for basal forearm plasma flow (F) and AAN arterio-deep venous difference (A-V) measured in the present study (Table I) and applying the Fick expression $\dot{Q} = F(A\text{-}V)$, one can compute the quantity, $\dot{Q}$, of amino acids released. This amounts to 1.07 μmoles/min per 100 g of forearm muscle (see Appendix). For a man weighing 70 kg whose skeletal muscle comprises 40% of body weight, 0.43 moles/day would be released from this tissue. This estimate agrees well with the total quantity of amino acids consumed by the liver as calculated from direct measurements of both hepatic urea output (36) and amino acid uptake (37) in postabsorptive man. Moreover, agreement is good between the pattern of individual amino acids released by muscle and their pattern of uptake by the liver (37, 38). If stoichiometrically converted by liver, these amino acids would generate approximately 40 g of glucose. Postaborptive hepatic glucose output is between 150 and 400 g/day (36, 39–41). Thus, amino acids delivered from the periphery play a quantitatively minor role as a source of glucose in the early postab-

sorptive period. Figures for urinary nitrogen excretion under these conditions support the contention that no more than 25% of the glucose released by liver derives from protein breakdown (42–44).

It has been known for a number of years that systemic insulinization, either by glucose or insulin infusion, lowers plasma levels of some amino acids while others are unaffected (45). Declines in threonine, isoleucine, leucine, tyrosine, phenylalanine, and valine are most consistently seen (46–49). Such changes, under the conditions studied, cannot be attributed to hormone effects on a specific tissue nor do they reflect conclusively a direct action of insulin. Changes in systemic insulin and glucose levels evoke compensatory changes in growth hormone, epinephrine, glucagon, and adrenal corticoids, all of which may alter nitrogen metabolism (50). In the present study, by close intraarterial infusion of insulin, forearm IRI was raised to postprandial levels in the absence of changes in systemic metabolite or IRI concentrations sufficient to evoke hormonal counterregulation. Insulin largely blocked the net output of amino acids from muscle, and probably from adipose tissue and skin as well. While most amino acids appeared to contribute to the 74% decline in total AAN output from muscle, consistent declines were seen only for threonine, isoleucine, leucine, tyrosine, phenylalanine, glycine, and α-aminobutyric acid. The close correspondence between those amino acids known to decrease systemically after systemic insulinization and those whose outputs from forearm muscle dropped after local insulinization in the present study suggest that systemic effects result from an action of insulin on muscle, and further, that factors controlling muscle amino acid release play a primary role in establishing and maintaining plasma levels. Supporting this is our observation that postabsorptive basal outputs of the various amino acids correlate well with ambient arterial levels. In our studies a net accumulation of amino acids in peripheral tissues was not observed even with maximum insulinization. Presumably, it is the combined effects of postprandial elevation of plasma amino acids coupled with the pancreatic insulin release which they stimulate (51) that lead to positive amino acid balance in peripheral tissues. Studies to test this hypothesis are in progress.

The failure of insulin to affect alanine release from forearm muscle along with the known resistance of plasma alanine level to change after systemic insulin administration deserve special comment. Evidence from direct measurements of splanchnic alanine extraction in man (37, 38) and from studies of its conversion to glucose in the perfused liver system (52) suggest that alanine is quantitatively the major nitrogenous glucose precursor. The absence of an insulin effect on alanine release in the present studies makes it unlikely that regulation of the supply of this amino acid substrate from muscle contributes substantially to insulin's overall antigluconeogenic action. Alanine is also released by the kidney in postabsorptive man (53). Quantitatively, however, this amounts to less than 25% of that released from muscle.

In our "low" dose insulin studies, blockade of FFA release from forearm adipose tissue when basal insulin levels were doubled confirms previous in vivo (31) and in vitro (54) work demonstrating extreme sensitivity of human adipocytes to insulin. Glucose arteriovenous difference across muscle increased in several subjects, and in others an increase could not be demonstrated, indicating that a doubling of basal insulin concentration approaches the threshold value for this biologic effect of insulin. Langs and Andres reported a small but reproducible increase in muscle glucose uptake when insulin was infused into the brachial artery at the same rate we have used for our "low" dose studies (32). In contrast, Zierler and Rabinowitz could demonstrate no change (31). Insulin readily binds to glassware and plastic tubing (30) accounting for the 63% loss we observed in its preparation and delivery. Slight differences in hormone loss during preparation and delivery could explain the disparities. Glucose uptake of striated muscle incubated in vitro is reproducibly stimulated by the addition of as little insulin as 50 μU/ml (55). The threshold of tissue sensitivity to stimulation of amino acid uptake in vitro is about the same (56). This agrees well with our observation that a doubling of the insulin level blocks net AAN output in some subjects but not in others. That these two actions of insulin, stimulation of glucose uptake and blockade of amino acid release, can occur independent of one another in man is suggested in the present study and has been proved in the in vitro situation.

APPENDIX

Total forearm flow is distributed partly to muscle and partly to subcutaneous adipose tissue and skin. Strictly speaking, the application of the Fick expression to calculate forearm muscle $\dot{Q}$ ($\dot{Q}_M$) requires knowledge of that fraction of flow supplying muscle (F_M), that is: $\dot{Q}_M = F_M(A\text{-}DV)$. The calculation of $\dot{Q}$ for amino acids made above is an incorrect estimate of $\dot{Q}_M$ only insofar as flow/100 g of muscle differs from that of an equivalent mass of superficial tissues. Previous workers have concluded, based on indirect evidence, that blood flow to muscle is greater per unit mass than to superficial tissue; hence, had the forearm been composed entirely of muscle, flow would have been 1.37 times as great. Consequently, they have multiplied $\dot{Q}$'s calculated as above by 1.37 to convert $\dot{Q}_T$ (total forearm $\dot{Q}$) to $\dot{Q}_M$ (22, 57). The assumptions pertaining to the derivation of this factor and their limitations have been previously discussed (22.) The argument hinges on the observations that in lean man muscle comprises 60% of forearm mass while it receives 82% of the blood flow (determined after epinephrine iontophoresis). In a recent study using the same technique of measuring total forearm flow before and after obliterating superficial flow by epinephrine iontophoresis, it has been found that as much as 50% of resting flow supplies

superficial tissue (58). Additionally, direct measurements of muscle (59) and subcutaneous adipose tissue (60) blood flow using a xenon^{133} wash-out technique have shown them to be about the same per unit mass (2.5 vs. 2.6 ml/min per 100 g). In view of this recent evidence suggesting, in fact, relative equality of blood flow to the various tissues, $\dot{Q}_T$ and $\dot{Q}_M$ are probably nearly identical and omission of the correction factor previously used seems reasonable.

ACKNOWLEDGMENTS

We wish to express our appreciation to Mrs. Elsa Vasmanis, Anna Karass, Marta Grinbergs, and Velta Ramolins for their meticulous technical work, and to Miss Terry Smith for her valuable nursing assistance.

This work was supported in part by U. S. Public Health Service Grants AM-05077, AM-09748, AM-09584, and the John A. Hartford Foundation, Inc., New York. Dr. Pozefsky is recipient of U. S. Public Health Service Special Fellowship 1F3-AM-36460.

REFERENCES

1. Pozefsky, T., P. Felig, J. S. Soeldner, and G. F. Cahill, Jr. 1968. Insulin blockade of amino acid release by human forearm tissues. *Trans. Ass. Amer. Physicians Philadelphia.* **81**: 258.
2. Pozefsky, T., P. Felig, J. S. Soeldner, and G. F. Cahill, Jr. 1969. Balance of individual amino acids across forearm muscle. *Clin. Res.* **17**: 393. (Abstr.)
3. Mirsky, I. A. 1938. The influence of insulin on the protein metabolism of nephrectomized dogs. *Amer. J. Physiol.* **124**: 569.
4. Frame, E. G., and J. A. Russell. 1946. The effects of insulin and anterior pituitary extract on the blood amino nitrogen in eviscerated rats. *Endocrinology.* **39**: 420.
5. Ingle, D. J., M. G. Prestrud, and J. E. Nezamis. 1947. The effect of insulin upon the level of blood amino acids in the eviscerated rat as related to the level of blood glucose. *Amer. J. Physiol.* **150**: 682.
6. Bollman, J. L., E. V. Flock, J. H. Grindlay, F. C. Mann, and M. A. Block. 1953. Action of glucose and insulin on free amino acids of the dehepatized dog. *Amer. J. Physiol.* **174**: 467.
7. Forker, L. L., I. L. Chaikoff, C. Entenman, and H. Tarver. 1951. Formation of muscle protein in diabetic dogs, studied with S^{35} methionine. *J. Biol. Chem.* **188**: 37.
8. Sinex, F. M., J. MacMullen, and A. B. Hastings. 1952. The effect of insulin on the incorporation of C^{14} into the protein of rat diaphragm. *J. Biol. Chem.* **198**: 615.
9. Krahl, M. E. 1953. Incorporation of C^{14}-amino acids into glutathione and protein fractions of normal and diabetic rat tissues. *J. Biol. Chem.* **200**: 99.
10. Akedo, H., and H. Christensen. 1962. Nature of insulin action on amino acid uptake by the isolated diaphragm. *J. Biol. Chem.* **237**: 118.
11. Manchester, K. L., and I. G. Wool. 1963. Insulin and incorporation of amino acids into protein of muscle. II. Accumulation and incorporation studies with the perfused rat heart. *Biochem. J.* **89**: 202.
12. Herrera, M. G., and A. E. Renold. 1960. Hormonal effects on glycine metabolism in rat epididymal adipose tissue. *Biochim. Biophys. Acta* **44**: 165.
13. Krahl, M. E. 1964. Stimulation of peptide synthesis in adipose tissue by insulin without glucose. *Amer. J. Physiol.* **206**: 618.
14. Andres, R., K. L. Zierler, H. M. Anderson, W. N. Stainsby, G. Cader, A. S. Ghrayyib, and J. L. Lilienthal, Jr. 1954. Measurement of blood flow and volume in the forearm of man; with notes on the theory of indicator-dilution and on production of turbulence, hemolysis, and vasodilatation by intra-vascular injection. *J. Clin. Invest.* **33**: 482.
15. Hoffman, W. S. 1937. A rapid photometric method for the determination of glucose in blood and urine. *J. Biol. Chem.* **120**: 51.
16. Moore, S., and W. H. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.* **211**: 907.
17. Trout, D. L., E. H. Estes, Jr., and S. J. Friedberg. 1959. Titration of free fatty acids in plasma: A study of current methods and a new modification. *J. Lipid Res.* **1**: 199.
18. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30**: 1190.
19. Soeldner, J. S., and D. Sloane. 1965. Critical variables in the radioimmunoassay of serum insulin using the double antibody technic. *Diabetes.* **14**: 771.
20. Morgan, C. R., and A. Lazarow. 1963. Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes.* **12**: 115.
21. Baltzan, M. A., R. Andres, G. Cader, and K. L. Zierler. 1962. Heterogeneity of forearm metabolism with special reference to free fatty acids. *J. Clin. Invest.* **41**: 116.
22. Andres, R., G. Cader, and K. L. Zierler. 1956. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. *J. Clin. Invest.* **35**: 671.
23. Fritz, I. B. 1961. Factors influencing the rates of long-chain fatty acid oxidation and synthesis in mammalian systems. *Physiol. Rev.* **41**: 52.
24. Rabinowitz, D., and K. L. Zierler. 1962. Role of free fatty acids in forearm metabolism in man, quantitated by use of insulin. *J. Clin. Invest.* **41**: 2191.
25. Waterlow, J. C., and J. M. L. Stephen. 1966. Adaptation of the rat to a low protein diet: the effect of a reduced protein intake on the pattern of incorporation of L-(^{14}C) lysine. *Brit. J. Nutr.* **20**: 461.
26. Thomas, L. W. 1962. The chemical composition of adipose tissue of man and mice. *Quart. J. Exp. Physiol. Cog. Med. Sci.* **47**: 179.
27. 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.
28. Andres, R., M. A. Baltzan, G. Cader, and K. L. Zierler. 1962. Effect of insulin on carbohydrate metabolism and on potassium in the forearm of man. *J. Clin. Invest.* **41**: 108.
29. Manchester, K. L. 1961. Insulin and incorporation of amino acids into protein of muscle. Cellular amino acid levels and aminoisobutyric acid uptake. *Biochem. J.* **81**: 135.
30. Weisenfeld, S., S. Podolsky, L. Goldsmith, and L. Ziff. 1968. Adsorption of insulin to infusion bottles and tubing. *Diabetes.* **17**: 766.
31. Zierler, K. L., and D. Rabinowitz. 1964. Effect of very small concentrations of insulin on forearm metabolism. Persistence of its action on potassium and free fatty

acids without its effect on glucose. *J. Clin. Invest.* **43**: 950.
32. Langs, H., and R. Andres. 1963. Insulin sensitivity of peripheral tissues in man. *Fed. Proc.* **22**: 443. (Abstr.)
33. London, D. R., T. H. Foley, and C. G. Webb. 1965. Evidence for the release of individual amino acids from resting human forearm. *Nature (London).* **208**: 588.
34. Kominz, D. R., A. Hough, P. Symonds, and K. Laki. 1954. The amino acid composition of actin, myosin, tropomyosin, and the meromyosins. *Arch. Biochem. Biophys.* **50**: 148.
35. Felig, P., T. Pozefsky, E. Marliss, and G. F. Cahill, Jr. 1969. Reduction of peripheral amino acid release: mechanism of decreased gluconeogenesis in prolonged starvation. *Diabetes.* **18** (Suppl. 1): 328. (Abstr.)
36. Bondy, P. K., D. F. James, and B. W. Farrar. 1949. Studies of the role of the liver in human carbohydrate metabolism by the venous catheter technic. I. Normal subjects under fasting conditions and following the injection of glucose. *J. Clin. Invest.* **28**: 238.
37. Felig, P., O. E. Owen, J. Wahren, and G. F. Cahill, Jr. 1969. Amino acid metabolism during prolonged starvation. *J. Clin. Invest.* **48**: 584.
38. Carlsten, A., B. Hallgren, R. Jagenburg, A. Svanborg, and L. Werkö. 1967. Arterio-hepatic venous differences of free fatty acids and amino acids. *Acta Med. Scand.* **181**: 199.
39. Myers, J. D. 1950. Net splanchnic glucose production in normal man and in various disease states. *J. Clin. Invest.* **29**: 1421.
40. Bearn, A. G., B. H. Billing, and S. Sherlock. 1951. Hepatic glucose output and hepatic insulin sensitivity in diabetes mellitus. *Lancet.* **2**: 698.
41. Werk, E. E. Jr., H. T. McPherson, L. W. Hamrick, Jr., J. D. Myers, and F. L. Engel. 1955. Studies on ketone metabolism in man. I. A new method for the quantitative estimation of splanchnic ketone production. *J. Clin. Invest.* **34**: 1256.
42. Benedict, F. G. 1915. A study of prolonged fasting. *Carnegie Inst. Wash. Publ.* 203.
43. Wilson, H. E. C. 1931. Studies on the physiology of protein retention. *J. Physiol. (London).* **72**: 327.
44. Cahill, G. F., Jr., M. G. Herrera, A. P. Morgan, J. S. Soeldner, J. Steinke, P. L. Levy, G. A. Reichard, Jr., and D. M. Kipnis. 1966. Hormone-fuel interrelationships during fasting. *J. Clin. Invest.* **45**: 1751.
45. Harris, M. M., and R. S. Harris. 1947. Effect of insulin hypoglycemia and glucose on various amino acids in blood of mental patients. *Proc. Soc. Exp. Biol. Med.* **64**: 471.
46. Crofford, O. B., P. W. Felts, and W. W. Lacy. 1964. Effect of glucose infusion on the individual plasma free amino acids in man. *Proc. Soc. Exp. Biol. Med.* **117**: 11.
47. Zinneman, H. H., F. Q. Nuttall, and F. C. Goetz. 1966. Effect of endogenous insulin on human amino acid metabolism. *Diabetes.* **15**: 5.
48. Carlsten, A., B. Hallgren, R. Jagenburg, A. Svanborg, and L. Werkö. 1966. Amino acids and free fatty acids in plasma in diabetes. I. The effect of insulin on arterial levels. *Acta Med. Scand.* **179**: 361.
49. Felig, P., E. Marliss, and G. F. Cahill, Jr. 1969. Hyperaminoacidemia: possible mechanism of hyperinsulinemia in obesity. *Clin. Res.* **17**: 382. (Abstr.)
50. Williams, R. H. 1968. Textbook of Endocrinology. W. B. Saunders Co., Philadelphia. 4th edition. 808.
51. Floyd, J. C., S. S. Fajans, J. W. Conn, R. F. Knopf, and J. Rull. 1966. Insulin secretion in response to protein ingestion. *J. Clin. Invest.* **45**: 1479.
52. Ross, B. D., R. Hems, and H. A. Krebs. 1967. The rate of gluconeogenesis from various precursors in the perfused rat liver. *Biochem. J.* **102**: 942.
53. Owen, E. E., and R. R. Robinson. 1963. Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. *J. Clin. Invest.* **42**: 263.
54. Gries, F. A., M. Berger, and K. Oberdisse. 1968. Untersuchungen zum antilipolytischen Effekt des Insulins am menschlichen Fettgewebe in vitro. *Diabetologia.* **4**: 262.
55. Vallance-Owen, J., and P. H. Wright. 1960. Assay of insulin in blood. *Physiol. Rev.* **40**: 219.
56. Manchester, K. L. 1965. Insulin and protein metabolism in muscle. *In* On the Nature and Treatment of Diabetes. B. S. Leibel and G. A. Wrenshall, editors. Excerpta Medica Foundation, Publishers. Amsterdam. 101.
57. Rabinowitz, D., and K. L. Zierler. 1962. Forearm metabolism in obesity and its response to intraarterial insulin. Characterization of insulin resistance and evidence for adaptive hyperinsulinism. *J. Clin. Invest.* **41**: 2173.
58. Wahren, J. 1966. Quantitative aspects of blood flow and oxygen uptake in the human forearm during rhythmic exercise. *Acta Physiol. Scand.* **67** (Suppl. 269).
59. Lassen, N. A. 1964. Muscle blood flow in normal man and in patients with intermittent claudication evaluated by simultaneous Xe^{133} and Na^{24} clearances. *J. Clin. Invest.* **43**: 1805.
60. Larsen, O. A., N. A. Lassen, and F. Quaade. 1966. Blood flow through human adipose tissue determined with radioactive xenon. *Acta Physiol. Scand.* **66**: 337.