Peripheral Tissue Metabolism in Cancer-bearing Man

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Whole-body tracer studies have documented abnormal glucose and amino acid kinetics in cancer-bearing man. Whether these abnormalities are related to systemic or local tumor effects is questioned. Forearm metabolism was examined in six patients with localized squamous cell carcinoma of the distal esophagus and six healthy normal male volunteers. Substrate arterio-venous differences and blood flow across forearm tissues were determined and substrate flux calculated. The mean forearm blood flow (ml min^{-1} 100 ml $forearm^{-1}$) was not significantly different between cancer patients (3.67 ± 0.12) and normal subjects (2.80 ± 0.40). The uptake of glucose (μmol min^{-1} 100 ml $forearm^{-1}$) was significantly higher in cancer patients (1.99 ± 0.45) compared to control subjects without weight loss (0.47 ± 0.18). Lactic acid release (μmol min^{-1} 100 ml $forearm^{-1}$) was significantly higher in cancer patients (−1.15 ± 0.35) compared to control subjects (−0.26 ± 0.14). There was no significant difference in the flux of individual amino acids between the groups, although the mean total nitrogen released from forearms of cancer-bearing patients was greater than that from normal controls. The arterial serum insulin level was significantly lower and the arterial plasma glucagon level significantly higher in cancer patients compared to control subjects. These data cannot be explained by weight loss alone and suggest a peripheral defect in metabolism in this group of cancer-bearing patients.

THE METABOLIC ALTERATIONS imposed on the host by the tumor-bearing state are varied and as yet ill-defined.[1-3] In animals, abnormalities in glucose metabolism, amino acid metabolism, and gluconeogenesis have been demonstrated, utilizing whole-body tracer studies.[4,5] Increased glucose turnover rate, increased rate of gluconeogenesis, and increased alanine turnover rate have been observed in tumor-bearing rats compared to non-tumor-bearing controls in the absence of significant cachexia.[4] The absence of cachexia is notable, since other investigators have attributed the abnormalities in gluconeogenesis and glucose metabolism to antecedent weight loss in tumor-bearing animals, and not to the cancer-bearing state.[6] Metabolic alterations similar to those observed in animals have been made by Waterhouse et al.[7] and Holyrode et al.[8] in cachectic cancer patients with metastatic disease. Increased glucose and alanine turnover rate and increased rate of gluconeogenesis have been documented in man with localized esophageal carcinoma,[9] compared to healthy controls.

The purpose of our study was to examine whether the above observations of abnormal whole-body glucose and amino acid kinetics were due to a localized tumor effect or due to abnormalities in peripheral metabolism imposed by the tumor-bearing state. Data to support a local effect are lacking in man, but in animals elegant studies have been performed demonstrating increased uptake of glucose and increased release of lactic acid by tumors *in vivo*.[10] Since comparable studies in man are technically difficult, we chose to study peripheral (forearm) tissue metabolism in a group of patients with localized squamous cell carcinoma of the esophagus. If the demonstrated metabolic abnormalities in man and animals were due to the local "tumor-trap phenomenon,"[11] then peripheral substrate metabolism, theoretically, should be reflective of the nutritional status and not of the tumor-bearing state *per se*.

Methods

Two groups of subjects were studied in an identical manner. The control group consisted of six normal male subjects with mean age 24.4 ± 4.0 (SEM) years and mean weight 66.4 ± 1.9 (SEM) kg. All normal subjects were carefully examined and had normal screening chemistry profiles and glucose tolerance tests. The cancer-bearing group consisted of five men and one woman with squamous cell carcinoma localized to the distal two-thirds of the esophagus. Patients were screened for evidence of metastatic disease by blood chemistries (including liver function tests), chest x-ray, chest computed tomography,

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Submitted for publication: April 28, 1982.

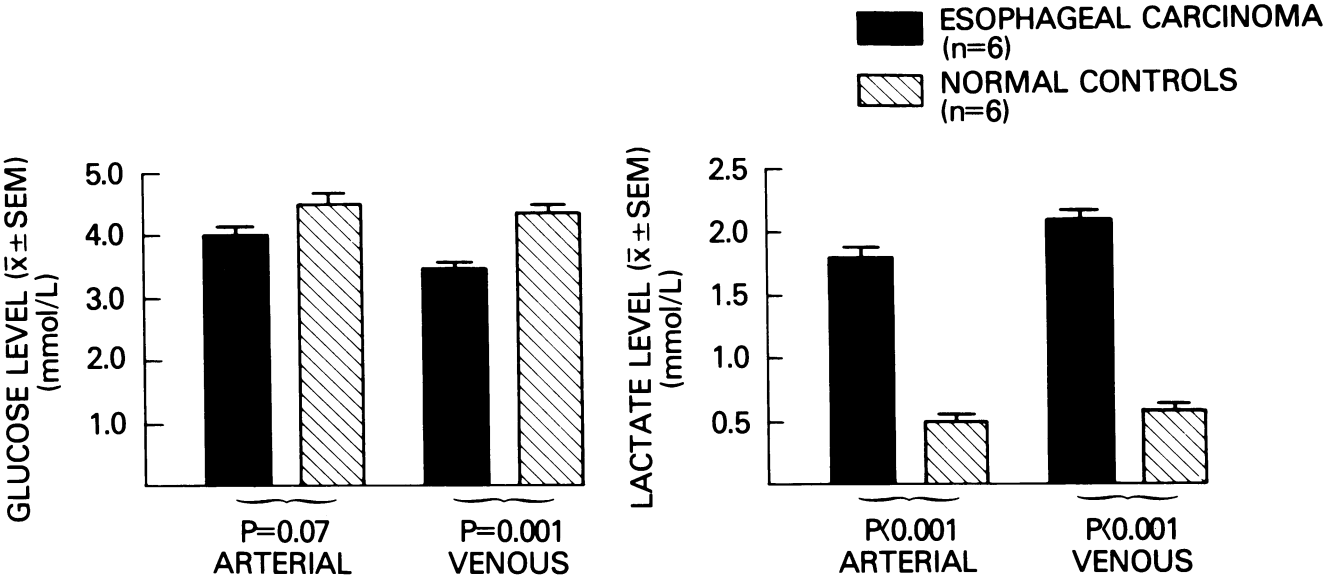

FIG. 1. Mean ($\bar{x}$) arterial and venous levels of glucose and lactate in six patients with esophageal carcinoma and six normal subjects.

liver and bone radionuclide scans, and liver ultrasonography. They were entered into the study if no evidence of metastases was documented. The mean age and weight of these patients was 65.3 ± 3.6 (SEM) years and 61.2 ± 2.4 (SEM) kg, respectively, and they had lost a mean of 19.9 ± 3.1 (SEM) % of their pre-illness body weight. Patients and controls read and signed informed consents approved by the Clinical Research Committees.

Postabsorptive control subjects and cancer patients had placement of plastic catheters into the nondominant radial artery and retrograde into the nondominant cubital deep vein on the morning of study. Previous studies have documented that venous blood obtained from proper placement of such catheters is from forearm musculature, with little or no contribution from subcutaneous or skin venous effluent.[12] All subjects rested quietly on a bed during the study. A pediatric blood pressure cuff was placed about the wrist and inflated to 250–300 mmHg 1–2 minutes before determination of forearm blood flow and blood sampling.

Blood flow was measured by capacitance plethysmography at the midforearm.[13–15] Venous occlusion was achieved with an adult blood pressure cuff applied about the arm and inflated rapidly to 40 mmHg. This method reliably and reproducibly measures blood flow and agrees with indirectly[16] and directly measured blood flow[17] in man.

Blood samples were obtained simultaneously from the radial artery and deep vein for determination of glucose[18,19] and lactic acid.[20,21] Arterial serum insulin[22,23] and plasma glucagon[24,25] were determined by radioimmunoassay. Whole blood amino acids were determined on a Beckman 121 MB® or 119 amino acid analyzer (Beckman Instruments, Inc., Fullerton, CA) after deproteinization with 10% sulfosalicylic acid (wt/vol) or 1.0 N perchloric acid.[26,27]

A positive arterial-venous difference will define uptake and negative will define release of a substrate. Flux is defined as the product of the blood flow multiplied by the arterial-venous difference.

All data are presented as the mean ± SEM. Statistical testing was done by Students's t-test (two-tailed) for unpaired data with unknown but assumed equal variance.[28] Statistical significance is defined as $p \leq 0.05$.

Results

Mean nondominant forearm blood flow (ml min^{-1} 100 ml $forearm^{-1}$) in six esophageal cancer patients (3.67 ± 0.12), although higher, was not significantly different from that in six normal control subjects (2.80 ± 0.40).

Mean arterial glucose level (mmol L^{-1}) was lower in the cancer patients (3.98 ± 0.15) compared to controls (4.47 ± 0.18), but not significantly so (Fig. 1). On the other hand, mean venous glucose level was significantly lower in the cancer patients (3.45 ± 0.11) compared to control subjects (4.32 ± 0.15). Both arterial and venous lactic acid levels (mmol L^{-1}) were significantly higher in the cancer patients (1.79 ± 0.09 and 2.11 ± 0.08, respectively) compared to the normal subjects (0.50 ± 0.04 and 0.58 ± 0.03, respectively) (Fig. 1). The arterial-venous (A-V) differences for glucose and lactic acid across the forearm were both significantly different in the cancer patients (0.53 ± 0.11 and −0.32 ± 0.10, respectively) compared to controls (0.15 ± 0.05 and −0.08 ± 0.05, respectively).

Forearm tissues in the cancer patients demonstrated a significantly increased uptake (μmol min^{-1} 100 ml fore-

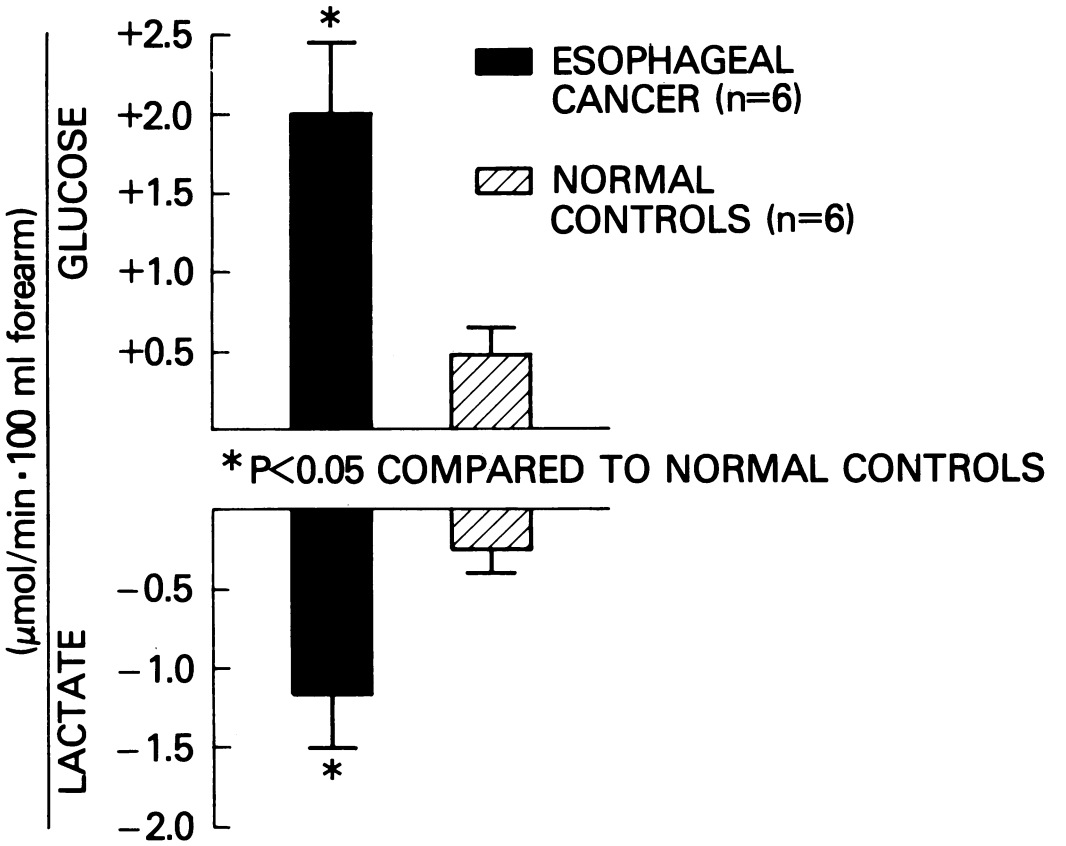

FIG. 2. Mean ($\bar{x}$) forearm flux of glucose and lactate in six patients with esophageal carcinoma and six normal subjects, (+) = uptake; (−) = release.

arm^{-1}) of glucose (1.99 ± 0.45) compared to control subjects (0.47 ± 0.18) (p = 0.01) (Fig. 2). The release of lactic acid (μmol min^{-1} 100 ml forearm^{-1}) was significantly greater from the forearm in the cancer patients (−1.15 ± 0.35) compared to control subjects (−0.26 ± 0.14) (p = 0.04).

TABLE 1. *Amino Acid Arterial Levels, Arterio-Venous (A-V) Differences and Flux Across the Forearms of Cancer Patients and Normal Controls (mean ± SEM)*

	Arterial Level (nmol ml^{-1})		A-V Difference (nmol ml^{-1})		Flux (nmol min^{-1} .100 ml forearm-1)	
Amino Acid	Cancer	Control	Cancer	Control	Cancer	Control
Taurine	180 ± 23	199 ± 15	8 ± 4	13 ± 11	30 ± 17	27 ± 30
Aspartic acid	139 ± 24	122 ± 8	9 ± 15	9 ± 6	28 ± 57	22 ± 16
Threonine	85 ± 8	125 ± 5*	−17 ± 7	−12 ± 5	−63 ± 28	−39 ± 16
Serine	107 ± 10	131 ± 6	−4 ± 5	3 ± 5	−14 ± 19	4 ± 11
Asparagine	43 ± 2	61 ± 4*	−10 ± 1	−7 ± 3	−38 ± 5	−23 ± 11
Glutamic acid	310 ± 11	179 ± 12*	3 ± 10	26 ± 5	11 ± 38	71 ± 9
Glutamine	137 ± 14	692 ± 10*	−19 ± 9	−42 ± 15	−73 ± 34	−135 ± 60
Proline	118 ± 9	207 ± 14*	−5 ± 8	−15 ± 6	−20 ± 34	−51 ± 23
Glycine	234 ± 23	285 ± 7	−12 ± 10	−10 ± 9	−45 ± 40	−37 ± 30
Alanine	133 ± 15	238 ± 8*	−53 ± 14	−42 ± 9	−195 ± 56	−101 ± 43
α-Aminobutyric acid	35 ± 3	19 ± 2*	3 ± 3	3 ± 1	12 ± 13	7 ± 4
Valine	253 ± 20	178 ± 17*	−5 ± 15	2 ± 8	−16 ± 59	1 ± 23
Cystine	89 ± 7	67 ± 19	6 ± 4	14 ± 12	22 ± 14	41 ± 15
Isoleucine	84 ± 7	51 ± 7*	4 ± 5	−1 ± 3	−14 ± 19	−4 ± 10
Leucine	159 ± 16	103 ± 4*	−7 ± 10	−8 ± 4	−23 ± 39	−26 ± 12
Tyrosine	41 ± 3	43 ± 2	−5 ± 2	−4 ± 1	−18 ± 10	−13 ± 5
Phenylalanine	44 ± 3	42 ± 3	−8 ± 3	−2 ± 3	−30 ± 13	−8 ± 9
Ornithine	85 ± 13	86 ± 6	4 ± 7	3 ± 4	14 ± 28	8 ± 9
Lysine	119 ± 7	146 ± 7*	−19 ± 2	−7 ± 8	−70 ± 47	−25 ± 21
Histidine	57 ± 4	77 ± 3*	−4 ± 4	4 ± 4	−16 ± 14	7 ± 10
Arginine	121 ± 19	70 ± 3*	1 ± 16	−3 ± 4	4 ± 61	−13 ± 13

* $p \leq 0.025$, unpaired t-test, cancer *vs.* control.

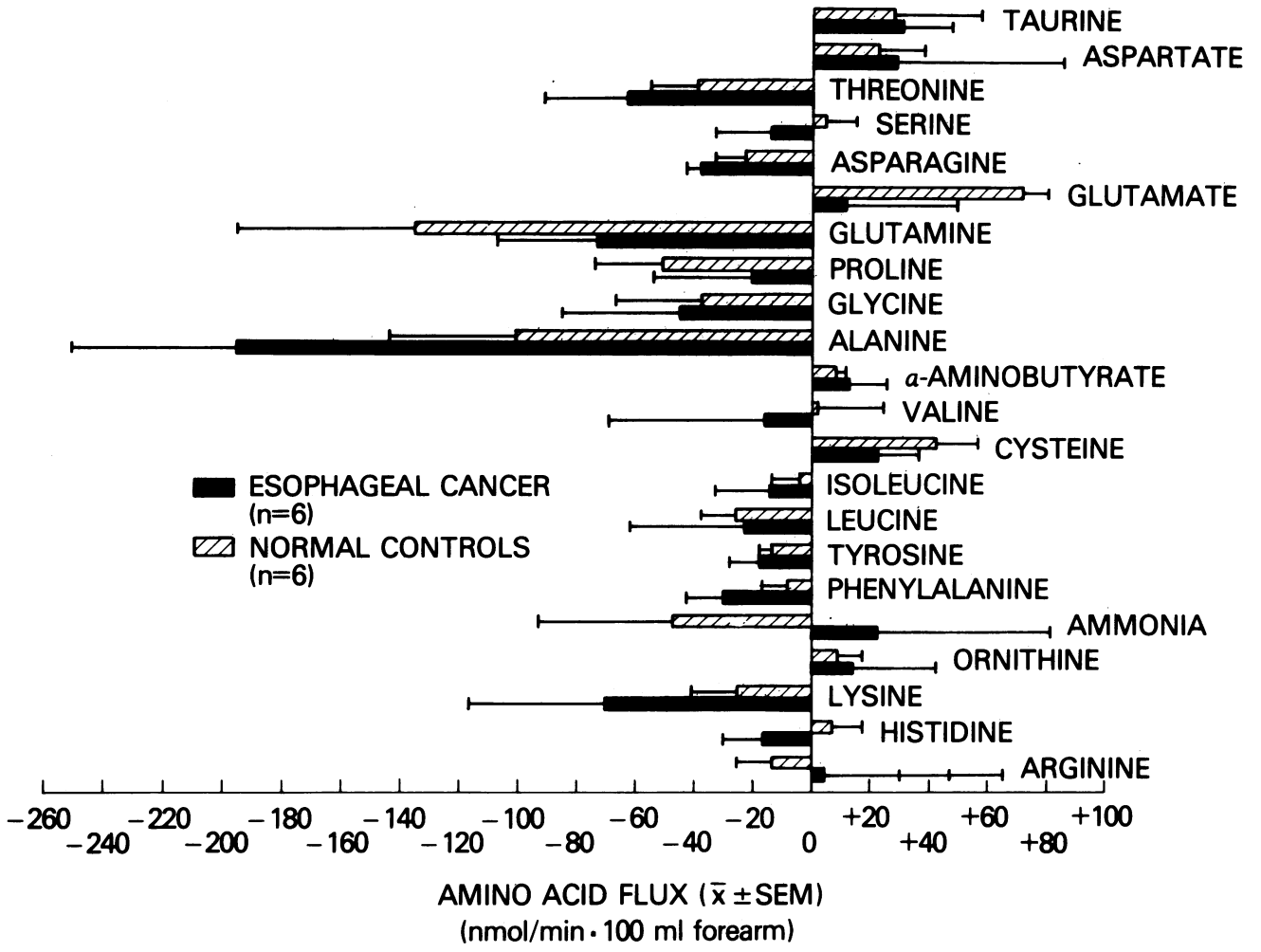

FIG. 3. Mean ($\bar{x}$) forearm flux of amino acids in six patients with esophageal carcinoma and six normal subjects, (+) = uptake; (-) = release.

The arterial whole blood levels of threonine, asparagine, glutamine, proline, alanine, lysine, and histidine were significantly lower, whereas those of glutamic acid, α-aminobutyric acid, valine, isoleucine, leucine, and arginine were significantly higher in cancer patients compared to control subjects (Table 1). There were no significant differences in whole blood amino acid arterial-venous difference or flux (Table 1, Fig. 3) between cancer patients or normal subjects. The pattern of amino acid flux pictured in figure 3 is very similar when esophageal cancer patients are compared to normal control subjects. The arterial ammonia levels (nmol ml^{-1}) were significantly greater in cancer patients (268 ± 21) compared to control subjects (99 ± 26), but there was no significant difference in ammonia flux (nmol ml^{-1} 100 ml $forearm^{-1}$) between the two groups (22 ± 59 *vs.* −47 ± 46) (Fig. 3).

Total nitrogen flux (nmol ml^{-1} 100 ml $forearm^{-1}$) was calculated from the amino acid flux for each individual and included ammonia but not urea. The cancer patients' (−651 ± 841) average release of nitrogen was approximately four times that of normal subjects (−165 ± 334). This was not significant.

Serum arterial insulin levels (μU ml^{-1}) were significantly lower and plasma glucagon (pg ml^{-1}) significantly higher in cancer patients (5.3 ± 1.3 and 110 ± 16, respectively) compared to control subjects (10.0 ± 1.0 and 28 ± 6, respectively).

Discussion

This study demonstrates significant alterations in forearm tissue metabolism in a group of middle-aged patients with localized esophageal cancer and weight loss when compared to young, normal subjects with no antecedent weight loss. If the abnormalities observed in the cancer patients are not due to the cancer-bearing state but due to the antecedent weight loss, then these data should be comparable to data from fasting volunteers. In obese,

normal volunteers fasted for 24 days, glucose uptake and lactate release by forearm tissues both decreased over the course of the fast.[29] If the differences observed in the cancer patients in this report, compared to the normal subjects, were due to relative starvation, one would expect the glucose uptake and lactate release from forearm tissues to be less, rather than greater than the normal controls. Therefore, the observed differences are probably not due to starvation.

It could be argued that the changes observed are not due to the cancer-bearing state, but secondary to the aging process, since the ages in our cancer patients and controls are disparate. Forearm tissue substrate flux data in older compared to younger patients are lacking, but studies in man looking at whole-body glucose turnover have demonstrated a significantly lower rate of glucose turnover in healthy elderly subjects (mean age 75 years) compared to healthy young subjects (mean age 24 years).[30] One might infer from the whole-body glucose turnover data that with increasing age, forearm tissue uptake of glucose would decrease. In this study, the cancer patients demonstrated a significant increase in glucose uptake, inferring that the observed difference is secondary to the cancer-bearing state and not due to age difference between the two groups.

That the changes observed in the cancer patients could be attributable to stress is debatable. The question of the cancer-bearing state as a stress model has been addressed,[1] but data are sparce. We did not measure catecholamine nor cortisol levels. Forearm data in mild stress or trauma conditions are lacking, but Long et al.[31] have demonstrated no significant changes in glucose turnover rate in five patients before and 2 days after surgery after major abominal operations. In seven patients with severe injury or sepsis, however, glucose turnover rate was double that of the controls. The degree of stress needed to produce significant changes in glucose metabolism is not apparent in our patients.

The results cannot be attributed to errors in the forearm blood flow measurement, the mean of which were not significantly different between the two groups. Even if the normal subjects had blood flow equal to that of the cancer patients, the difference in flux of glucose and lactate between the two groups would still be greater than 100%.

If one then accepts the postulate supported by this report and others[1,3,7-9] that cancer induces a distinct set of metabolic alterations in man that differs from "pure starvation," this study aids in defining the mechanism of the metabolic pertubation. Although previous *in vivo* tracer studies have demonstrated an increase in the whole-body glucose turnover rate, alanine turnover rate, and rate of gluconeogenesis from alanine in cancer-bearing man,[7,9] the mechanism for these alterations was not addressed. The data available from animal studies would support a local tumor effect. Gullino et al.[10] demonstrated marked glucose uptake and lactic acid release from a variety of transplantable rat tumors grown on a single arterio-venous pedicle. This observation would support a local pertubation secondary to the tumor. The host response to this increased "peripheral glucose utilization" by the tumor would be increased hepatic glucose production by gluconeogenesis and glycogenolysis. In animals, the *in vivo* whole-body tracer studies would also support this concept.[4] As yet, there are few data to corroborate this hypothesis of a local "tumor trap" mechanism in animals, as the only mechanism for the metabolic alterations observed since *in vivo* studies of peripheral tissue metabolism in cancer-bearing animals compared to nontumor-bearing controls are lacking.

In man, a similar mechanism has been thought to explain the increased whole-body rates of gluconeogenesis, glucose turnover, and alanine turnover observed.[7,9] Supportive evidence for this is available in a study by Norton et al.[32] This study demonstrated increased uptake of glucose across soft-tissue sarcoma-bearing limbs compared to the control (nontumor-bearing) limb in man by measuring blood flow by capacitance plethysmography and the glucose arterio-venous difference across the respective limbs. In this small group of patients, there was a significant correlation between the tumor limb glucose uptake and the postamputation measured size of the tumor. Again, as in the animal work, there are few data to support the concept that the whole-body metabolic alterations in cancer-bearing man are due simply to a local tumor effect with counter-regulatory host response.

The present study supports the concept that the metabolic alterations associated with the tumor-bearing state are not simply a function of a local tumor effect, but that there is also a systemic component. If the simplistic local effect was all that was operative, then peripheral glucose uptake and lactic acid release would tend to be unaffected, or possibly decreased. In this study, forearm tissues in the cancer patients demonstrated significantly increased glucose uptake and increased lactic acid release compared to normal subjects. The mechanism for these observations is unclear. Since the serum insulin levels were significantly less in the cancer patients compared to controls, increased insulin cannot be implicated, but the presence of nonsuppressible insulin-like activity (NSILA) might be an explanation.[33] NSILA has been implicated as the cause of hypoglycemia observed with some nonislet cell tumors in man.[34] Although the source of NSILA is not documented, most investigators favor production and excretion by the tumor.[35] Increased NSILA levels might explain the increased forearm muscle uptake of glucose in the cancer patients reported here, leading to the lower blood glucose levels observed and subsequently the lower serum insulin level, as an appropriate response to decreased glu-

cose concentration. In response to the decreased blood glucose level, the liver would increase glucose production, predominantly by gluconeogenesis. Overall, this would result in an increased whole-body glucose turnover rate and an increased rate of gluconeogenesis, as has been observed in the cancer patient.[7,9]

The increased lactate release by forearm tissues observed in the cancer patients is probably secondary to the increased utilization of glucose and not a primary event. The increased lactic acid release by peripheral tissues probably accounts for the increased arterial whole-blood lactate levels demonstrated in our cancer patients. In addition, this increased flux of lactate probably contributes to the increased whole-body Cori cycle activity demonstrated in cancer-bearing man utilizing *in vivo* tracer techniques.[36]

In obese subjects, fasting for 40 days leads to significantly lower arterial plasma levels of valine, leucine, isoleucine, and arginine.[37] In this study, the arterial blood levels of these four amino acids were significantly higher in the cancer patients compared to normal subjects; again supporting the existence of a specific tumor-bearing metabolic state distinct from starvation. The arterial levels of proline, alanine, and histidine in the cancer patients were significantly lower than normal controls and this change was also observed in prolonged starvation.[37] Although differences were observed for arterial levels of amino acids, there was no significant difference noted for flux of any amino acid measured between our two groups. This is probably secondary to the large variation in flux noted for each amino acid and the small number of subjects in each group. This same reasoning may explain the large mean difference between total nitrogen flux observed, with no significant difference between groups demonstrated. The cancer-bearing group released approximately four times as much total nitrogen as the normal subjects. This finding is probably unrelated to either the increased plasma glucagon[38,39] or to the decreased serum insulin level[40] observed in the cancer patients, but probably secondary to increased liver uptake of amino acids for gluconeogenesis.[7,9]

In summary, this study demonstrates abnormal peripheral tissue (forearm) metabolism in a group of patients with localized squamous cell carcinoma of the distal esophagus and weight loss compared to normal subjects. Evidence from this study and others quoted previously would tend to support the concept that there exist metabolic abnormalities that are tumor-specific and not related to antecedent weight loss. It appears from animal and human studies that there may be two factors operating to initiate the changes observed. The first would be marked, obligate utilization of glucose by the tumor, the tumor "glucose trap" phenomenon. Secondly, peripheral tissues appear to be stimulated to utilize increased amounts of glucose, perhaps by tumor secretion of humoral substances, such as NSILA or other growth factors. The combined effect would increase whole-body glucose utilization, which would tend to decrease plasma glucose levels and, in turn, serum insulin levels. In order to maintain homeostasis, the host increases glucose production primarily by increased gluconeogenesis, Cori cycle activity, and glycogenolysis. The overall effect is to increase the whole-body glucose turnover rate and rate of gluconeogenesis. The peripheral tissues in turn demonstrate increased release of gluconeogenic precursors, principally in the form of amino acids. These changes would tend to deplete peripheral tissues of protein to supply substrates for both the tumor and the host and may contribute to the cachexia observed in cancer.

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