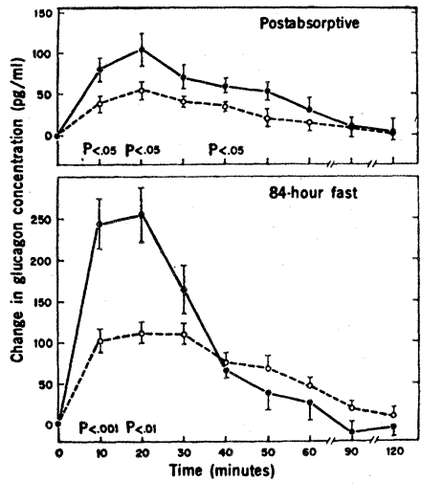

Fig. 1. Changes in glucagon concentration in plasma from basal amounts in nonobese and obese subjects infused with alanine when they were in the postabsorptive state, and after an 84-hour fast. Results shown represent the means ± standard error. *P* values indicate significance of differences between nonobese and obese subjects. Solid line, nonobese subjects; dotted line, obese subjects.

weight subjects, the relative importance of such factors as obesity per se, prolonged alterations in dietary intake, and hyperinsulinemia cannot be determined from the present data. However, the higher concentrations of glucose in the blood of the obese group while they were starved may contribute to the diminished alpha cell response observed at that time.

The demonstration that obese individuals are capable of maintaining glucose homeostasis during starvation in the absence of an elevation in concentration of glucagon in the plasma suggests than an increase in secretion of this hormone is not essential for glycogenolytic and gluconeogenic processes induced by starvation. The question may be raised as to whether the high incidence of reactive hypoglycemia reported in obese subjects (*15*) may be related to altered alpha cell function.

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Synthesis of Cysteine from Methionine in Normal Adult Subjects: Effect of Route of Alimentation

Abstract. *Parenteral alimentation solutions free of cysteine, probably an essential amino acid for premature infants, were administered continuously to eight healthy men through catheters in the superior vena cava and through nasogastric tubes. When the preparation was administered parenterally, the plasma cystine concentration dropped markedly. When feeding was switched to the oral route, the concentration rose immediately, but returned to baseline only when a cystine-containing diet was fed. These studies indicate that the synthesis of cysteine from methionine is limited, even in the adult subject, when cystine-free diets are administered parenterally.*

The administration of high-calorie solutions by central venous catheter to infants and adults who are unable to take an adequate diet orally has gained wide acceptance. Such intravenous preparations supply an adequate amount of calories in the form of dextrose and sufficient quantities of a protein hydrolyzate to achieve positive nitrogen balance and net protein synthesis.

These solutions given by the central venous route bypass the liver and the gut, two important sites in the control of plasma amino acid concentrations. When solutions are administered orally, the liver has direct access to dietary amino acids through the portal circulation. These ingested amino acids and peptides, together with liver and plasma proteins, comprise an important part of the labile protein reserve. The liver can alter the concentration of amino acids in portal blood before entry into peripheral circulation and can supply or utilize amino acids rapidly in a manner complementary to the needs of other tissues, properties important to the homeostatic control process (*1*). One example of such complementary interaction has been noted for the amino acid alanine during starvation and exercise (*2*). The gut also is important in amino acid metabolism, affecting such factors as stereospecificity of amino acid absorption, rapid absorption of peptides and their conversion to component amino acids before release into the portal circulation, interconversions of certain amino acids, degradation and synthesis of amino acids by the intestinal flora, the length of time a meal is available for absorption, and interactions due to the metabolic activity of the tract itself (*3*).

The nitrogen sources of solutions designed for total parenteral alimentation in this country are usually protein hydrolyzates of casein or beef fibrin, and contain approximately equal quantities of free amino acids and peptides. Stegink and Baker (*4*) showed that the amino acid composition of these hydrolyzates directly affects amino acid concentrations in plasma, and noted that these concentration changes could be logically explained when the amino acid composition of each nitrogen source was considered. In particular, the absence of cystine in such preparations was reflected by low plasma cystine and taurine concentrations in infants maintained by parenteral feeding. They suggested that the composition of amino acid solutions infused

should be adjusted so that normal amino acid concentrations are maintained during infusion, because the infused solution enters the circulation directly without first passing through the gut and liver. This is of special concern in the parenteral feeding of newborn or premature infants. Snyderman's studies of nitrogen balance and growth (*5*) indicate that cystine (or cysteine) and tyrosine are essential amino acids for the premature infant; their absence from the diet caused depressed plasma concentrations as well. Sturman *et al.* (*6*) demonstrated that L-homoserine hydrolyase (deaminating) (E.C. 4.2.1.15) is absent (or present in low activity) in the liver of the human fetus or the premature or newborn infant liver and suggested that cysteine (or cystine) is an essential amino acid at these ages.

To further study the factors controlling plasma amino acid concentrations in normal subjects during parenteral alimentation, we compared the influence of the route of alimentation of such amino acid mixtures on plasma cystine concentrations. Eight healthy adult male volunteers were admitted to the clinical research center. After receiving a baseline general diet for 3 days, the subjects were fed fat-free diets eucalorically (calorie intake sufficient to maintain constant weight and a slightly positive nitrogen balance). Seventy percent of calories were derived from glucose and 30 percent from an amino acid hydrolyzate devoid of cystine or cysteine (Amigen); the composition is listed in Table 1. The solution (1 kcal/ml) was given by continuous infusion through either a catheter in the superior vena cava or a nasogastric tube. Feeding by each route was administered for 2 weeks. The two feedings were in sequence and in random order. The weights of the subjects varied less than 1.5 kg during the entire 4-week study. Plasma samples were obtained at 8 a.m. daily to avoid circadian rhythm effects, and were processed immediately. Protein was removed with solid sulfosalicylic acid (*7*), and samples were analyzed in Technicon NC-1 amino acid analyzers as described previously (*4*). These conditions avoid loss of cystine in plasma samples (*8*).

The results of these experiments are shown in Fig. 1. When the preparation was administered parenterally, plasma cystine concentrations dropped markedly within 12 hours and remained depressed for the entire 2-week period. When the identical solution was

Table 1. Composition of protein hydrolyzate infusion (content per liter).

Substance	Amt.	Substance	Amt.
	(g)		(meq)
Protein*	40	Na^+	30
Glucose	260	K^+	15
Lipid	0	Cl^-	20
	(mg)	Phosphate	24
Folic acid	37	Ca^{2+}	12.5
Ascorbic acid	500	Mg^{2+}	4.2
Thiamine HCl	25		(units)
Riboflavin	10	Vitamin A	10,000†
Niacinamide	100	Vitamin D	1,000†
Pyridoxine HCl	20	Vitamin E	5‡
Panthenol	25		

* Amino acid composition is given in (*4*). † U.S. Pharmacopeia units. ‡ International units.

then administered by nasogastric tube, plasma cystine concentrations rose immediately to 3.5 μmole per 100 ml and remained near that value for the next 2-week period. Plasma cystine concentrations returned to baseline (5.5 μmole per 100 ml) only when a cystine-containing diet was fed. The order in which the feedings were administered had no effect (Fig. 1).

Cystine is not considered an essential amino acid for the human adult, because early studies indicated that it could be synthesized in adequate amounts from methionine (*9*). Rose and Wixom (*10*) were able to maintain adult subjects in positive nitrogen balance for at least 8 days when they were fed diets devoid of cystine, a result indicating adequate cystine synthesis. They also noted (*11*) that 80 to 90 percent of the dietary methionine requirement could be replaced by cystine, which suggests that much of the required methionine is used for cystine synthesis. However, the oral route of administration does affect cystine synthesis, as we have shown in these studies.

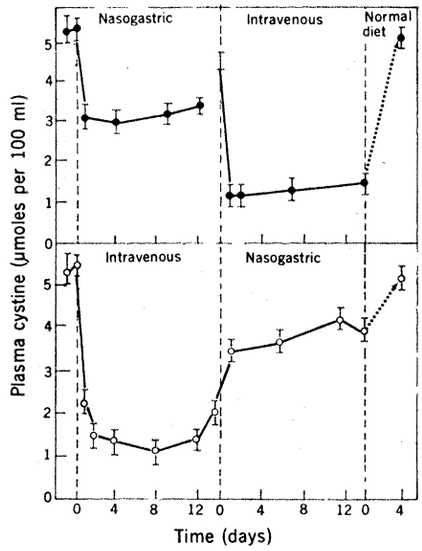

Fig. 1. Plasma cystine concentrations (mean ± standard deviation) in normal male subjects given parenteral alimentation solution. Solid circles are data for subjects fed first by nasogastric tube and then by intravenous infusion; the order of feeding routes was the reverse for other subjects (open circles).

An altered plasma amino acid pattern is the most reproducible and extensively studied biochemical change in experimental animals fed an imbalanced diet devoid of an essential amino acid. In general, plasma concentrations of the amino acid in question are depressed, and tissue concentrations are usually similarly affected (*12*). Our data thus suggest a possible imbalance involving cystine in parenteral alimentation preparations.

Our data indicate that individual amino acids behave in different ways depending on the route of infusion. Amigen solution is high in glutamate and devoid of glutamine, whereas plasma is relatively high in glutamine and low in glutamate. The infusion of this solution through the superior vena cava did not result in increased plasma glutamate concentrations; this indicates that the peripheral tissues are able to adequately metabolize glutamate.

In contrast to the rapid metabolism of infused glutamate, synthesis of cysteine from orally administered methionine appears to be somewhat limited even though the entire amino acid intake passes through the liver by means of the portal circulation. Our data show that this synthetic capability is even less when the cystine-free diet is fed parenterally, a result suggesting that extrahepatic tissues have limited capacity to carry out this conversion. However, such limited synthesis may be adequate for normal tissue maintenance even though it is not sufficient to maintain normal plasma concentrations. The increased plasma cystine concentration noted during oral administration of the parenteral alimentation products points out the importance of considering the role of the gut during the design of solutions for parenteral alimentation. The increase in the plasma cystine concentrations during oral administration may reflect the importance of the gut in the utilization of the peptide material present in these preparations. Peptides appear to be absorbed more rapidly from the gut than are the component amino acids of such peptides (*3*). Little

information is available, however, concerning utilization of peptide material during parenteral administration of these products. These data suggest that the effect of bypassing the gut and liver during parenteral alimentation must be considered in designing future solutions for parenteral feeding of both the young infant and the adult.

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Murine Leukemia Virus: Restriction in Fused Permissive and Nonpermissive Cells

Abstract. *Cultures of human cells nonpermissive for mouse leukemia virus replication could not be induced to support virus replication by homologous fusion in the presence of Moloney leukemia virus. Human cells were also fused with permissive mouse cells, and the fate of the virus in heterokaryons was determined by a simultaneous autoradiography and fluorescent antibody technique. Heterokaryons containing the full chromosome complement of both cells were likewise nonpermissive for virus synthesis, but hybrids of human and mouse cells, which lacked up to half of the human chromosome complement, were permissive for virus synthesis. The results suggest that human cell genes can direct a repressive control over mouse leukemia virus replication.*

Evidence of variable expression of mouse RNA tumor-virus antigens in vivo has led to speculation about host regulation of virus expression (*1*). While some strains of mouse sarcoma viruses transform cultured human cells (*2*), few human cell strains have been reported to be permissive for mouse leukemia virus replication (*3*). We have tested for host-cell control of virus expression by examining the nature of cellular restrictions for the replication of mouse leukemia virus by nonpermissive human cells. Cellular restrictions for the replication of several other viruses have been investigated by cell fusion and by the use of hybrids of permissive and nonpermissive cells (*4–7*). With two exceptions (*7*) the results of such studies have shown that restriction at the cell membrane, or the absence of a virus-required function, could be overcome by fusion or by hybrid formation which rendered the nonpermissive cells competent to support virus replication. In contrast, our results show that nonpermissive human cells can restrict mouse leukemia virus replication even in the presence of a full chromosome complement from the permissive mouse cell.

The 3T3-M cell strain, which is permissive for Moloney leukemia virus (*8*) was used between passages 4 and 30 in our laboratory. Human WI-38 cells (*9*) were used between passages 15 and 35. The cell cultures were grown in Eagle's minimum essential medium with 5 to 10 percent fetal calf serum, were maintained without antibiotics, and were free of *Mycoplasma* contamination.

The sensitivity of WI-38 cells for Moloney leukemia virus (*10*) was tested by infecting DEAE-dextran-treated cells (*11*) with the virus at concentrations sufficient to infect up to 60 percent of the 3T3 cells in the first replication cycle. The WI-38 cells were completely restrictive, as determined by fluorescent-antibody (*12*) and XC (rat cells transformed by Rous sarcoma virus) cell-plaque (*13*) techniques. Since the restriction could represent the absence of specific cell receptors for the virus, we attempted to induce infection of WI-38 cells with Moloney leukemia virus by fusion with inactivated Sendai virus. The cells were treated with DEAE-dextran prior to fusion; virus replication, assayed by the techniques cited above, could not be detected when tested up to 4 days later (Table 1, group A).

Since the mechanisms of cell penetration by the RNA tumor viruses is not clearly known, we attempted to determine if C-type virions could be detected intracellularly in the fused cells by electron microscopy. At intervals after the initiation of fusion, the cells were removed from the plates by scraping; they were then fixed in glutaraldehyde, packed by centrifugation at about 2000*g*, imbedded, sectioned, and examined with the electron microscope. We were unable to detect intracellular particles with the typical C-type morphology, but electron-dense structures with a diameter of 40 to 90 nm were seen, and their frequency and distribution indicate that they could be C-type virus nucleoids in various stages of uncoating (*14*).

Three primary alternatives may account for the nonpermissive state of human cells. First, the cells may lack specific virus receptors. In this case, if the fusion process permitted incorporation of the virus, replication should have been detected in at least a small percentage of cells. Second, the cells could be deficient for functions required for virus replication. Third, the cells could exert a repressive control over some viral functions. In order to test the last two alternatives, we examined the fate of the virus in heterokaryons of WI-38 and 3T3 cells. The WI-38 cells were specifically labeled during growth in medium containing [^{3}H]thymidine (0.5 μc/ml). Heterokaryons were formed by fusion of equal numbers of each cell type with inactivated Sendai virus immediately after the mixed cells had been treated with DEAE-dextran and exposed to Moloney leukemia virus. When viewed with ultraviolet light through a 470-nm barrier filter, both the fluorescent antibody and autoradiographs could be viewed simultaneously (*15*), so that the heterokaryons and cells synthesizing viral protein could be determined (Fig. 1, A and B).

The results (Table 1) showed that heterokaryons of WI-38 and 3T3 were all negative for viral synthesis, while