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Amino Acid Metabolism in Mammalian Cell Cultures

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A number of cell lines derived from normal and malignant tissues have now been serially propagated in culture. These may be grown adherent to a glass surface and overlaid with a fluid medium, or they may be grown in suspension; but in either case the opportunity is presented for the study of metabolism at the cellular level in a system which operationally resembles bacterial cultures in most essential respects. The cells and the medium can be separately analyzed, balance experiments can be set up, metabolic processes can be examined qualitatively and quantitatively under controlled conditions, and the corresponding enzymatic activities can be explored in cell-free extracts.

A relatively limited number of metabolites have been shown to suffice for the apparently indefinite propagation of all the human cell lines so far studied. The minimal medium, in which every component is demonstrably essential, is listed in Table 1 and includes 29 components: 13 amino acids, 8 vitamins, 6 ionic species, glucose, and serum protein (*1, 2*). The role of the serum protein is not entirely clear. Although a few cell lines have been serially propagated in a protein-free medium (*3*), serum protein is required by most mammalian cell cultures. In monolayer cultures, the protein plays a role in the adhesion of the cells to glass (*4*). However, its essentiality in suspension cultures indicates that this is not its only function, and it seems reasonable to assume that it acts in part as a carrier of as yet unidentified growth factors which are bound to the protein, and which are slowly released into the medium.

The present article, dealing with the amino acid metabolism of these cultured cells, is a progress report rather than a review and in large part summarizes studies from a single laboratory. The enormous body of information available with respect to amino acid metabolism in bacterial cultures (*5, 6*) has served as a stimulus and prototype for the studies here reported. The relevant findings in bacterial systems have not been referred to in detail, only because that important exercise in comparative biochemistry is beyond the scope of the present report.

Nutritionally Essential Amino Acids

The classic experiments of Rose and his coworkers (*7*) have shown that eight amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) suffice for nitrogen balance in feeding experiments in man. In contrast, every cell culture so far examined, whether human or animal (*8*) in origin, and whether deriving from normal or malignant tissue (*9*), has required at least 13 amino acids for survival and growth. Over and above the eight amino acids required for nitrogen balance, these cell cultures require arginine, cyst(e)ine, glutamine, histidine, and tyrosine, and on the omission of any one of these, the cells degenerate and die. In their early stages these degenerative processes are reversible. This presents an opportunity to explore the intimate structural derangement caused by specific amino acid deficiences, and the reparative processes which occur on restoration of these amino acids to the medium.

None of the D-amino acids substitute for the L-isomer (*8*) except for D-cystine, and this apparently acts by mobilizing cyst(e)ine residues bound to the serum protein of the medium. (This is discussed below.) Dipeptides were found to be active, substituting for both component amino acids (*10*). Recent experiments indicate that, although dipeptides are hydrolyzed extracellularly by serum peptidases, and perhaps by cell-derived peptidases, some of the dipeptide is transported into the cell and there hydrolyzed.

A number of keto acids and other amino acid congeners have been tested with respect to their ability to substitute for the corresponding amino acid (*11*). The results with several human and animal cell lines are summarized in Table 2. The cells contain a wide variety of transaminases (*12*), and, as determined in feeding experiments in rats (*5*), most of these keto acids do in fact substitute for the corresponding amino acid.

Of particular interest is the fact that, with all the human cell lines studied, citrulline substituted for arginine, while ornithine was inactive [Table 2; see also (*13*)]. Attempts to encourage citrulline biosynthesis by progressive removal of

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citrulline from a medium containing ornithine have to date been unsuccessful.

A number of possibilities may be considered in explanation of the fact that at least five amino acids which are not required for nitrogen balance are nevertheless essential for the survival and growth of cells in culture.

1) *Loss of biosynthetic mechanisms during prolonged growth in vivo.* The enzymatic systems which, in vivo, permit the biosynthesis of arginine, cyst(e)ine, glutamine, histidine, and tyrosine may have been lost, in whole or in part, during the hundreds of generations of rapid growth in vitro. To assess this possibility, cells deriving from monkey kidney, monkey testis, and rabbit embryonic tissues were examined with respect to their amino acid requirements in first culture passage, within 24 to 48 hours after their isolation from the animal host and prior to extensive multiplication (*11, 14*). All 13 amino acids required by long-term cell cultures were necessary also for the survival and growth of these first-passage cells. The requirement for 13 amino acids rather than eight is therefore not due to loss of biosynthetic mechanisms in the course of prolonged growth in culture, but these experiments do not exclude the possibility that the necessary enzymes are lost from the cells within the first 48 hours after their removal from the animal.

2) *Lack of appropriate precursors or cofactors (the role of folinic acid in glycine synthesis).* A second possibility is that cultured cells retain the capacity to synthesize these five amino acids but that this is not evidenced in culture for lack of appropriate precursors or cofactors. This possibility is illustrated in the biosynthesis of glycine by monkey kidney cells in primary culture. For optimum growth, these cells require glycine (*14, 15*) because of a partial block in the conversion of serine to glycine (*16*), and that glycine requirement has been shown to be eliminated by the provision of folinic acid (*17*). The primary block in the biosynthesis of glycine by this cell culture is apparently in the reduction of folic acid to the metabolically active cofactor. However, no simple precursor has yet been found which can be used by cultured cells in lieu of preformed arginine, cyst(e)ine, glutamine, histidine, or tyrosine, and no cofactor has been recognized which, as in the case of folinic acid and glycine, will stimulate that biosynthesis by cultured cells.

3) *Limited biosynthetic capacity, sufficient for survival but not for growth.* As a third possible explanation for the essential nutritional role of arginine, cyst(e)ine, glutamine, histidine, and tyrosine in cell cultures, it is possible that all mammalian cells, both in vivo and in vitro, have a limited capacity for the biosynthesis of these amino acids which could suffice for maintenance and nitrogen balance in whole-animal feeding experiments but which is inadequate for the rapid growth characteristic of cell cultures. Such partial synthesis has been demonstrated under certain conditions for glutamine, cyst(e)ine and tyrosine.

Glutamine synthesis. Although glutamic acid at physiological levels usually does not substitute for glutamine in these cell cultures, experiments with C^{14}-labeled glutamic acid and N^{15}-ammonia have shown (*18*) that the cells do have a limited capacity to make glutamine from these precursors. Since glutamic acid, in turn, is formed to only a limited extent from glucose, glutamine is an essential amino acid for all of the serially propagated human cell cultures so far studied. However, monkey kidney cells in primary passage can use glutamic acid or glutamine interchangeably (*14*), and in these cultures the minor biosynthesis of glutamic acid from glucose permits prolonged survival, and occasionally limited growth, in a medium lacking both glutamine and glutamic acid. A similar limited biosynthesis by human cells could account for the fact that glutamine is not necessary for nitrogen balance in short-term feeding experiments in man.

Although it is not relevant to the main thread of this discussion, it is of interest to note that with every cell line so far examined, high levels of glutamic acid substitute for glutamine (*19*). DeMars has made the important observation that at these high concentrations the cells form greatly increased amounts of glutamine synthetase (*20, 21*). This increased enzyme formation was product-inhibited—that is, the formation of glutamine synthase was inhibited by the presence of glutamine. Having formed the enzyme, the cells can then grow at low levels of glutamic acid for long periods, and perhaps indefinitely (*21*). This may be the first case of enzyme induction in serially propagated mammalian cell cultures.

Monkey kidney cells in primary culture, which can utilize glutamic acid and glutamine interchangeably, were found to have a glutamine synthetase activity

Table 1. Minimum essential medium* for cultivation of mammalian cells in either monolayer or suspension. The concentrations of most of the amino acids are greater than those originally recommended (*1*) and the relative amounts conform more closely to the protein composition of cultured human cells (*23*). This permits the cultures to be kept for somewhat longer periods without refeeding. The concentration of some of the salts has been rounded off. In using this medium for the growth of cells in suspension, Ca^{++} should be omitted or greatly reduced in order to minimize clumping (see *43*), and the concentration of NaH_2Po_4 may be increased tenfold for more effective buffering (*43*).

Compound	Concentration (m*M*)	Concentration (mg/lit.*)	Compound	Concentration (m*M*)	Concentration (mg/lit.*)
L-Amino Acids			*Salts*		
Arginine	0.6	105	NaCl	116	6800
Cystine	0.1	24	KCl	5.4	400
Glutamine*	2.0	292	$CaCl_2$	1.8 (0)†	200 (0)†
Histidine	0.2	31	$MgCl_2 \cdot 6H_2O$	1.0	200
Isoleucine	0.4	52	$NaH_2PO_4 \cdot 2H_2O$	1.1 (11)†	150 (1500)†
Leucine	0.4	52	$NaHCO_3$*‡	23.8	2000†
Lysine	0.4	58	*Vitamins**		
Methionine	0.1	15	Choline		1
Phenylalanine	0.2	32	Folic acid		1
Threonine	0.4	48	Inositol		2
Tryptophan	0.05	10	Nicotinamide		1
Tyrosine	0.2	36	Pantothenate		1
Valine	0.4	46	Pyridoxal		1
*Carbohydrate**			Riboflavin		0.1
Glucose	5.5	1000	Thiamine		1
			Serum Protein§		
			Whole or dialyzed serum, 5 to 10%		

* The fact that some of the amino acids can be replaced by such immediate precursors as the corresponding keto acids (*11*), that the vitamins can be replaced by the corresponding cofactors (*44*), or that a number of carbohydrates will substitute for glucose (*45*) does not change the minimum number of essential growth factors but relates only to the form in which they may be supplied. Conversely, the fact that bicarbonate, for example, is not an essential nutrilite for any of the serially propagated lines so far examined has no bearing on its essential metabolic role and means only that CO_2 is formed in adequate amounts from other components of the minimal medium—for example, glucose.

† In suspension cultures (see *43*).

‡ With small cell populations, the concentration of $NaHCO_3$ may be reduced from 0.20 to, for example, 0.04 percent in order to minimize the alkalinization of the medium in the early stages of growth.

§ Optional supplementation is as follows: (i) "nonessential" amino acids (alanine, asparagine, aspartic acid, glycine, glutamic acid, proline, serine), each at 0.1 m*M*; (ii) sodium pyruvate (1 m*M*). Of these, asparagine, serine, glycine, and pyruvate have proved necessary for the growth of certain cell lines, in a dialyzed serum medium, and serine is similarly required for the growth of single cells.

Table 2. Growth response of cultured cells to keto acid analogs of essential amino acids and related compounds (*11*).

Amino acid	Keto acid analog*	Growth response† of		
		Human cell lines‡	Mouse cell lines§	Rats (*5*)
L-Histidine	β-Imidazolyl-pyruvic	++	++	+
L-Isoleucine	α-Keto-β-methylvaleric	++	++	+
L-Leucine	α-Ketoisocaproic	++	++	+
L-Methionine	α-Keto-γ-methiolbutyric	++	++	+
L-Phenylalanine	Phenylpyruvic	++	++	+
L-Tyrosine	*p*-Hydroxyphenylpyruvic	±		+
L-Valine	α-Ketoisovaleric	++	++	+
L-Arginine	α-Keto-δ-guanidinovaleric	0	0	0
L-Threonine	α-Keto-β-hydroxybutyric	0		
L-Tryptophan	β-Indolylpyruvic	0		+
Other analogs substituting for amino acid in growth of human cell lines				
Arginine	Citrulline	+ to ++		
Histidine	Imidazole lactic acid	±		

* Other inactive analogs are as follows. Arginine: α-ketocarbamidovaleric acid, L-ornithine, guanidine, guanidoacetic acid. Histidine: L-histidinol, L-histidinol phosphate, imidazole glycerol, imidazole glycerol phosphate, imidazole acetol phosphate. Leucine: leucic acid. Lysine: DL-pipecolic acid. Methionine: DL-homocystine (+ serine), DL-homoserine (+ cystine or inorganic sulfur). Valine: α-hydroxyisovaleric acid.
† (++) Growth rate of cultures equal to that obtained with amino acid: (+) growth rate approximately half that observed with amino acid; (±) definite but slight growth; (0) no significant growth response.
‡ HeLa, KB, conjunctiva, liver, Detroit 116P.
§ Mouse fibroblast (strain L-929) and sarcoma 180.

of the same order of magnitude as that of glutamic-acid–adapted HeLa cells (*22*).

Provision of cyst(e)ine in a "cystine-free" medium. Four general pathways for the provision of cyst(e)ine have proved to be used by cultured human cells (*2*), including *de novo* biosynthesis. That biosynthesis alone does not, however, suffice for growth.

One pathway involves the release of preformed cysteine from cystathionine. L-Homocystine is also active, but only in part by virtue of the condensation of homocysteine with L-serine to give L-cystathionine. In addition, as is discussed in a following paragraph, it serves to dissociate cysteine residues bound to the serum protein of the medium. The degree to which methionine can be used to synthesize cysteine by way of homocysteine is under present study.

Cultured human cells contain a large pool of taurine and glutathione (*23*). When they are grown in a cyst(e)ine-free medium, that glutathione, unlike the taurine, largely disappears (*11*). Its quantitative utilization for the provision of cystine would permit a 50- to 75-percent increase in cell protein. It remains to be seen whether, as in *Escherichia coli* (*24*), there is a similar cannibalization of dispensable cell proteins.

Table 3. The biosynthesis of the "nonessential" amino acids. The numbers in the body of the table represent (i) specific activity per C atom, or (ii) atoms percent excess, in each case referred to that of the precursor as 100, it being assumed that all incorporation is into newly synthesized protein. (After *17, 18*).

Amino acid synthesized by cell	Labeled precursors in medium						
	Glucose-U-C^{14}	Serine-U-C^{14}	Glutamine-U-C^{14}	Glutamic-U-C^{14}	Glutamine-amide N^{15}	Glutamic-N^{15}	$N^{15}H_3$
	Carbon skeleton				Amide N	α-NH_2	
Alanine	76	.65	7	7		80	4
Glycine	77	66		7		75	5
Serine	78	65	2	10		76	4
Asparagine					84		
Aspartic	11	.66	68	58		78	6
Glutamine					100		
Glutamic	6	.08	62	84		83	4
Proline	7	.26	39	27		58	
Cyst(e)ine	22–47	22–32		2			

The serum protein which must be added to the medium for the sustained growth of almost all mammalian cell lines (*1*) binds significant amounts of amino acids, which are released on boiling or on treatment with trichloroacetic acid (*11*). Bound cyst(e)ine is present in dialyzed human and horse serum at a concentration of at least 0.03m*M*, and the slow but continuing growth which may be observed in a cyst(e)ine-free medium (the rate increasing with the concentration of serum) is due in part to the mobilization of this bound cyst(e)ine. The marked stimulatory effect of reduced inorganic sulfur compounds (S^{--}, $S_2O_3^{--}$, $S_2O_4^{--}$, SO_3^{--}) on cell growth in a cyst(e)ine-free medium is similarly due to the fact that they promote the release of the bound cystine residues from the serum protein (*11*). As shown by experiments with $Na_2S^{35}O_3$, the inorganic S is not itself used in cyst(e)ine biosynthesis (*11*), unlike the case of *E. coli* (*24*). D-Cystine and D-homocystine also promote growth in a cystine-free medium, as will glutathione, thioglycollate, cysteamine, and cystamine (*11*). These compounds mobilize bound cyst(e)ine from serum protein; in addition, some may yield NaSH by the action of a cellular desulfhydrase.

Over and above the utilization of preformed cystine, or of closely related compounds such as glutathione and cystathionine, the cell can synthesize limited amounts of cyst(e)ine from glucose (*11*) (see Table 3). The source of the sulfur in this limited biosynthesis, which of itself does not suffice for survival and growth, is probably methionine, by way of homocystine.

Biosynthesis of tyrosine from phenylalanine. There is another example of a limited amino acid biosynthesis which might suffice for nitrogen balance and maintenance but which does not suffice for the sustained growth of cell cultures. A HeLa cell variant appeared spontaneously, characterized by a fibroblast-like appearance, which overgrew the parent strain. This variant culture, unlike any other cell line so far examined, was able to effect the hydroxylation of phenylalanine to tyrosine to a minor but significant degree (*25*). The reaction was completely inhibited in the presence of tyrosine—another example of a product-inhibited amino acid biosynthesis. Despite their ability to make significant amounts of tyrosine, these altered cells could not survive in a tyrosine-free medium, and added tyrosine was necessary for survival and growth. None of the other cell lines so far ex-

amined have been able to effect this hydroxylation, even in trace amounts, at least under the conditions of these experiments and in the medium of Table 1.

4) *The possible physiologic significance of the requirement of 13 amino acids.* In explaining the fact that cultured cells require arginine, cyst(e)ine, glutamine (or a precursor amino acid), histidine, and tyrosine, a final possibility may be considered: that most body cells do in fact require all five of these amino acids. It could be that they are unnecessary for nitrogen balance in feeding experiments in man only because they are supplied either by the action of the intestinal flora on the other amino acids or by the metabolic action of, for example, the liver.

Biosynthesis of the "Nonessential" Amino Acids

Of the amino acids which are nutritionally nonessential, the carbon skeletons of three (alanine, serine, and glycine) derive primarily from glocose, while four (asparagine, aspartic acid, glutamic acid, and proline) derive their carbon primarily from glutamine (*17, 18*). This dissociation is, however, not complete (see Table 3). In a medium containing both glucose and glutamine, 2 to 7 percent of the alanine and serine carbons derive from glutamine, and, conversely, amino acids of the glutamine family derive 5 to 11 percent of their carbon from products of glucose metabolism. The biosynthesis of cyst(e)ine is discussed above.

The specific source of the α-amino nitrogen of the eight nutritionally nonessential amino acids is now under study. Glutamic acid, either directly or by way of aspartic acid, is apparently the primary source of the α-NH_2 group of alanine, serine, glycine, asparagine, and proline. Free ammonia, which derives almost entirely from the amide group of glutamine, when cells are grown in the minimal medium of Table 1, is used by the cells to only an insignificant degree for the biosynthesis of either amino acids, purines, or pyrimidines (*18, 26–28*). (The only exception is the biosynthesis of glutamine from glutamic acid.)

Particular interest attaches to the biosynthesis of asparagine. In a minimal medium its carbon skeleton has been found to derive from glutamine, presumably by way of aspartic acid, while its amide nitrogen derives primarily from the amide nitrogen of glutamine (*27*). There is no present information as to the exact metabolic steps involved.

Vitamin B_6 has been found to play an essential role in the synthesis of alanine, serine, glycine, and proline (*29*). In cells depleted of pyridoxal, the concentration of these amino acids in the cell pool was markedly reduced (*30*), and growth, which ceases in the vitamin-deficient cell, could be restored by the addition to the medium of these nonessential amino acids (*29, 30*).

The designation of eight amino acids as nutritionally nonessential requires qualification on several scores. As indicated in Table 4, at least one cell line has shown a requirement for asparagine (*31*) over and above the requirement for glutamine. A rabbit fibroblast has been found to require serine (*32*), as did cells from the limb-buds of 22-day-old rabbit embryos (*11*), and monkey kidney cells in primary culture require glycine for optimal growth (*14, 15*).

There is another situation in which normally nonessential amino acids regularly become essential for the optimum growth and even for the survival of serially propagated cells. The minimal medium of Table 1, supplemented with 3- to 5-percent dialyzed serum, suffices for the indefinite growth of all the serially propagated human cell cultures so far examined, provided that a sufficiently large inoculum is used. However, when a minute cell population is placed in a relatively large volume of fluid, as in cloning experiments (*33*), that basal medium is usually no longer effective. The rate of growth of the clones is markedly reduced, and the cloning efficiency—that is, the proportion of cells which grew out to form visible colonies—is sometimes also greatly reduced (*34*). The growth of these small cell populations has been shown to be restored to normal levels by the addition either of whole serum, of higher concentrations of dialyzed serum, or of the nonessential amino acids (*34*). In most of the experiments the complete mixture of the nutritionally nonessential amino acids could be replaced by a single amino acid, serine. Glycine was usually only partially effective, and this probably reflects the limited degree to which glycine can be used by these cells for the biosynthesis of serine (*16*).

Preliminary results indicate that the major factor involved in this anomalous requirement for a "nonessential" amino acid, serine, by small cell populations is the loss of serine from the cell pool into the medium at a rate which exceeds the biosynthetic capacity of the cell (*26*).

Amino Acid Pool

All the cultured human cells examined to date have been found to contain a significant amino acid pool (*23*), the composition of which is essentially similar to that in animal tissues. Glutathione, taurine, glutamine, ammonia, and glutamic acid are present in largest amounts and together constitute approximately 60 percent of the total pool amino acids.

At concentrations in the medium comparable to those in the body fluids,

Table 4. Nutritional and metabolic differences in cell cultures.

Cell strain	Unusual nutritional requirement	Unusual metabolic activity	Reference
Walker carcinosarcoma (256)	Asparagine		(*31*)
Rabbit fibroblast (RM3) and embryonic limb-bud cells	Serine		(*17, 32*)
Monkey kidney cells in primary culture	Glycine	Aspartic } Glutamic } → glutamine	(*14, 15*) (*14*)
Mouse fibroblast (strain "L")		Biosynthesis of inositol in amounts sufficient for growth	(*46*)
Mouse leukemia (strain 388)	Pyruvate		(*47*)
Mouse embryonic cells	Pyruvate		(*11*)
HeLa "variant"		Phenylalanine → tyrosine	(*25*)
HeLa clones	Varying concentrations of serum protein		(*33*)
Small populations of HeLa cells (S3)	"Nonessential" amino acids (notably serine) required for optimum growth		(*34*)

on the order of 0.1m*M*, most of the nutritionally essential amino acids are concentrated by the cell approximately five- to tenfold. The degree to which the amino acid can be concentrated by the cell does, however, vary with the environment—from, for example, as much as 200-fold at an outside concentration of 0.001m*M* to as little as twofold at an outside concentration of 10m*M* (*26*). The rate of transport into the cell is quite rapid, usually reaching 75 percent of the equilibrium value within 15 minutes, and the loss of amino acid when the cells are placed in an amino-acid-deficient environment is correspondingly rapid.

It has been possible to determine for a number of specific amino acids the average intracellular concentration necessary for the initiation of protein synthesis and cell growth (*17*). When the pool concentration of a given amino acid was less than 0.01m*M*, there was usually no net synthesis of protein or cellular multiplication; maximum rates of protein synthesis and cell growth were observed at pool concentrations of 0.1 to 0.2m*M*, and at intermediate levels the rate of cellular growth varied directly with the amino acid concentration.

Metabolism of the Essential Amino Acids

All but one of the nutritionally essential amino acids so far studied (phenylalanine, tyrosine, lysine, valine, and threonine) have been found to be metabolized by the cell to only a minimal degree. Their primary function in cell cultures appears to be their utilization for incorporation into protein (*25, 35*). Glutamine, however, is actively metabolized. Over and above its direct utilization for protein synthesis, it is converted to glutamic acid, aspartic acid, asparagine, and proline and, to a limited degree, to serine and alanine as well. Its carbon skeleton is heavily used in the biosynthesis of pyrimidines, presumably by way of aspartic acid, and in the biosynthesis of both purines and pyrimidines, the glutamide amide nitrogen contributes more than half of the total base nitrogen (*28*). Its role in poliovirus synthesis is discussed below.

Protein Turnover

When cultures are overlaid with a medium lacking one or more essential amino acids, there is of course no net synthesis of protein. Isotopically labeled amino acids are nevertheless incorporated from such media into the cell protein at the surprisingly high rate of approximately 0.7 to 1 percent per hour (*25, 35*). This rate is uniform for all the cell lines and amino acids studied and may continue for as long as 72 hours, by which time more than 50 percent of the specific amino acid residues of the protein have been replaced. The reverse process (that is, the loss of amino acids from prelabeled cell protein) proceeds at the same rate, provided that the corresponding amino acid is present in the medium. This incorporation is largely an intracellular process and is only in small part due to either cell degeneration or protein secretion into the medium, followed by degradation to the amino acid level and resynthesis (*35*).

In animal cells, there is as yet no conclusive evidence as to whether this intracellular replacement of amino acid residues in cell protein involves the total breakdown of proteins to the amino acid level, followed by their resynthesis, or whether it is instead an exchange reaction between free amino acids in the cell pool and corresponding residues in an otherwise intact, or only partially degraded, protein molecule. Unlike the case in bacteria (*36*) and yeast (*37*), the turnover process occurs at essentially the same rate in growing as in nongrowing cultures, and is there evidenced by incorporation of a labeled amino acid into protein in amounts exceeding that represented by the new protein synthesized (*25, 35*). It is noteworthy that metabolic inhibitors such as azide, dinitrophenol, chloramphenicol, 6-mercaptopurine, and 5-fluorodeoxyuridine, at concentrations which totally inhibit the net synthesis of protein, have no significant effect on the rate of protein turnover (*17*).

Amino Acids in Biosynthesis of Poliovirus

In heavy monolayer cultures of the HeLa cell, the biosynthesis of poliovirus has been shown to require only glucose, glutamine, and salts (*38*); for the rest, the precursors for virus were derived from the cell itself. That study left open the question as to whether the viral protein was formed from the amino acid pool of the cell, whether the cells utilized their own protein for viral synthesis, or whether these two possibilities were the same, in the sense that cell protein turnover could supply the necessary free amino acids. Recent observations indicate that the synthesis of poliovirus by the HeLa cell requires the presence of a full complement of amino acids in the pool.

In contrast to the results obtained in large cell populations, when a relatively small number of cells were placed in a large volume of medium containing only glucose, glutamine, and salts, the amount of virus formed per cell was strikingly reduced (*39*). In this situation there is a rapid loss of amino acids from the cell pool (*17*); and the capacity of the cell to form virus was restored by the addition of a full complement of amino acids to the medium (*39*). It is clear that the optimal poliovirus synthesis by the HeLa cell requires the presence of free amino acids in the pool, and it is a reasonable presumption, borne out by recent experiments with labeled amino acids and purified virus (*40*), that these amino acids are used for the synthesis of viral protein.

It is of interest that the concentrations of glucose and of glutamine necessary for the optimal synthesis of virus were the same as those required for cell growth. Glutamic acid substituted for glutamine in virus propagation at the same high and nonphysiologic levels which permitted cell growth, and glutamic-adapted cells, which could grow at low levels of glutamic acid by virtue of their increased capacity to synthesize glutamine, formed maximal amounts of poliovirus at those same low levels (*41*).

General Considerations

Provision of the 28 essential metabolites outlined in Table 1, supplemented with serum protein, permits the large-scale cultivation of a wide variety of serially propagated cell lines in both monolayer and suspension cultures, at an average generation time in the logarithmic phase of growth of 18 to 24 hours. The same minimal medium, supplemented with serine, permits the growth of single human cells in 10 milliliters of culture fluid with 50 to 100 percent cloning efficiency. Some animal cell lines require further specific supplementation (see Table 4). Although this basal medium has been used successfully for the cultivation of cells directly from the animal host (*42*), there is no information as to the proportion of the cells which grow out, or as to the additional growth factors which would be required for maximally efficient cloning. Until such cloning can be achieved with essentially 100 percent efficiency, the identification of cell cultures as, for ex-

ample, "liver," "lung," or "bone marrow" is suspect. It is further to be noted that specialized organ functions are conspicuously absent in almost all serially propagated dispersed cell cultures. It remains to be determined whether this reflects incorrect identification of the cells, an irreversible loss of their biosynthetic capacities, absence from the environment of necessary precursors or cofactors, or the fact that cellular organization and interaction are essential to those specialized functions.

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Robert Emerson, Investigator of Photosynthesis

At the present state of knowledge of biology the experimental approach, designed to establish empirical relations between relevant variables, usually turns up unexpected effects and thus serves as the starting point for the development of entirely new concepts. Robert Emerson was particularly adept at this type of exploratory measurement uncommitted to any particular theory. His life was spent on experimental investigation of the mode of action of pigments in green plant photosynthesis. The effect of his career, directly on the field of photosynthesis and indirectly on related aspects of biology, is of particular value not only because of his own discoveries but also because he established unusually high standards of performance and critical self-evaluation of experimental measurements.

Born in 1903 to Haven Emerson (later to be public health commissioner for New York City as well as professor of public health at Columbia University) and a Philadelphia Quaker mother, he grew up in New York, attended the Ethical Culture School, and spent his summers in rural Long Island. He graduated from Harvard in 1925 and received his doctorate in 1927 from the University of Berlin; his work for the doctorate was based on studies of *Chlorella* respiration, made in collaboration with Otto Warburg. At this time he isolated the "Emerson strain" of *Chlorella pyrenoidosa,* which became the standard plant for photosynthesis research. He returned to Harvard for two years as a National Research Council fellow, and there he taught a course on photosynthesis and experimented with the effects of light intensity, temperature, and chlorophyll content on photosynthesis of *Chlorella.* In his lectures be made experimental data speak for themselves —a powerful technique that he developed to a high level of effectiveness.

In 1929 he married Claire Garrison and moved to California Institute of Technology. There, with the assistance of William Arnold, he investigated the effects of flashing light on photosynthesis. These experiments are the basis for the concept of the "photosynthetic unit" —a group of chlorophyll molecules functioning together as a single entity. This concept turned out to be very fruitful and continues to provide incentive for many new experiments and discussions.

Emerson's scientific work reflected his extraordinarily forceful character. Two characteristics that influenced the course of his research and left a vivid impression on his students and colleagues were his strong moral sense and his outrage at sloppy performance of any sort. His desire to do everything with excellence resulted in clear-cut, definitive scientific results. No relevant details of experi-