23. V. K. La Mer, L. A. G. Aylmore, T. W. Healy, *J. Phys. Chem.* **67**, 2793 (1963).
24. M. L. Robbins and V. K. La Mer, *J. Colloid Sci.* **15**, 123 (1960); V. K. La Mer and M. L. Robbins, *J. Phys. Chem.* **62**, 1291 (1958).
25. E. J. Benton (*11*), p. 235.
26. F. H. C. Steward, *Australian J. Appl. Sci.* **2**, 157 (1960).
27. R. G. Vines, R. J. Meakin, *ibid.* **10**, 190 (1959).
28. J. T. Davies and E. K. Rideal, *Interfacial Phenomena* (Academic Press, New York, 1961), pp. 265–274.
29. U.S. Bureau of Reclamation, *Chemical-Engineering Lab. Rept. No. SI-39* (1 Jan. 1962).
30. Oral communication to contractors at Bureau of Reclamation meeting, Feb. 1963.
31. L. O. Timblin, Jr., Q. L. Florey, W. U. Garstka (*11*), p. 177.
32. V. K. La Mer, *Natl. Acad. Sci. U.S. Publ. 942* (1963), p. 438.
33. R. G. Vines, *Wool Technol. Sheep Breeding* **6**, 137 (1960); *Australian J. Phys.* **13**, 45 (1960); *Australian J. Appl. Sci.* **11**, 157 (1960).

Metabolic Controls in Cultured Mammalian Cells

Cultured cells may provide a direct approach to regulation and function in the whole animal.

Harry Eagle

With the widespread experimental use of animal cell cultures, data are accumulating on the factors which control their metabolic activity. Although that information is incomplete and large areas remain essentially unexplored, a summary and assessment may nevertheless be timely. Of particular interest is the relevance of the in vitro findings to metabolic processes in the whole animal. Cultured animal cells may divide every 16 to 30 hours, while the generation time in the whole animal is more often on the order of 30 to 90 days. What is the growth controlling mechanism from which the cultured cell has escaped almost wholly, and from which the cancer cell in vivo has escaped in small part? Can the study of metabolic controls in discrete cultured cells in fact be expected to throw light on their growth in the whole animal, to say nothing of even more complicated phenomena such as differentiation and morphogenesis, senescence, and the malignant transformation? Such processes involve cells of diverse types complexly organized into tissues, in which intercellular effects and spatial orientations difficult to reproduce in culture play an important and perhaps decisive role.

The specific aspects of metabolic controls in cultured cells here discussed have been selected, not because of a single unifying theme, but simply because of their relevance to the work interests of this laboratory. The topics include (i) the possibility that the provision of nutrilites may limit or control growth in vivo; (ii) some of the factors which control or modify the specific enzymatic activities of cultured cells; (iii) the profound effects of cellular interaction on their metabolic activities, with particular reference to population density and contact; (iv) genetically determined variations in metabolic activity, including inborn errors of metabolism, "variant" cells, and the effects of chromosomal aberrations; (v) the metabolic effects of viral infection; and (vi) some of the factors which control the expression of specialized organ function in cultured normal cells. In each case, the mass of published data precludes a complete bibliographic citation (*1*).

Nutritional Controls

The simplest method of controlling the growth and metabolism of cultured cells is to limit the supply of some essential nutrilite. Only 28 growth factors have been shown to be required for the sustained growth of mammalian cells in vitro (*2*), and through control of the input of one or more of these, the average generation time can be varied from 16 hours to 7 days (*3*). Thus, if the intracellular pool of a single amino acid falls below a critical concentration of 0.01 to 0.04 m*M*, there is no demonstrable protein synthesis or cellular growth, and a slight increase in pool size then has an altogether disproportionate effect on the rate of cellular proliferation (*4*). We do not know which of the sequential steps involved in protein synthesis is the growth-limiting reaction controlled by the size of the cellular amino acid pool.

It is, however, unlikely that the local provision of amino acids, glucose, or vitamins limits and controls the rate of cellular growth in a living animal on an adequate diet. Most of these growth factors are present in the body fluids in concentrations far exceeding those required for maximum growth in vitro, and there has been no evidence that cells in tissues differ from cultured cells in their ability to transport and concentrate amino acids, for example (*5*). There is, however, one unknown in the nutrition of animal cells which may conceivably be growth-limiting in the whole animal. Almost all cultured cells require one or more substances deriving from serum protein for survival and growth. (The fact that a small number of cell lines can dispense with the protein-derived factor, or factors, as the result of either adaptation or selection does not affect the general statement.) The active component is apparently not the protein as such, but a relatively small molecule, either a polypeptide or a substance bound to the protein and released from it (*6, 7*), the provision of which may be growth-limiting in vivo. It seems more likely, however, that cellular growth in the whole animal is controlled, not by the supply of an essential nutrilite, but by as yet unknown factors which actively limit or stimulate cellular growth and division. Although growth-inhibitory factors may develop in cell cultures (*3*), and although growth-inhibitory effects are sometimes observed when different cells are grown parabiotically, we know nothing about the nature of the compounds involved, the mechanism of their action, or their relevance to growth control in vivo.

The author is chairman of the Department of Cell Biology at the Albert Einstein College of Medicine, Bronx, New York. This article is adapted from lectures presented 2 June 1964 at the Baylor University College of Medicine, Houston, Texas, and 12 December 1964 at the New York University School of Medicine, New York, N.Y.

Enzyme Activity

Immediate effects of explantation. The very initiation of a dispersed cell culture is attended by profound alterations in the cell's metabolic activity, for it is suddenly exposed to an environment which differs in two major respects from the situation in vivo. The cells are deprived of a wide spectrum of compounds essential for growth and function which are normally supplied in the body fluids, and which the specific cell either cannot synthesize at all or can synthesize only in small amounts. In addition, the population density decreases drastically, from approximately 10^8 per milliliter in parenchymatous tissues to a level of 10^4 to 10^6 per milliliter. The 100- to 10,000-fold dilution leads to the elution of materials which the cell can synthesize, but which it must retain at metabolically effective levels in order to grow or to function: either compounds used as such for macromolecular synthesis, cofactors required for enzymatic activity, or the enzymes themselves. It is therefore not surprising that the plating efficiency of cells freshly isolated from tissue may be 0.1 percent or less, and that many cell types have yet to be cultured. The surprising feature is, rather, that any cells can be grown in vitro in the face of this drastic environmental change.

The initial lag in the establishment of a culture, during which time cells are adapting to the conditions of in vitro growth, may last from a few hours to many days, depending on the specific tissue, the cell type, and the population density, and perhaps on the growth factors which are provided over and above the minimum essential requirements which suffice for most established cell strains. The profound changes in metabolic activity which take place during this initial period of acclimation are illustrated by marked changes in a wide variety of enzyme activities (*8*), the usual disappearance of differentiated organ function, and the disappearance of organ-specific antigens (*9*).

Controls of enzyme activity in established cultures. Even after hundreds of generations in culture, certain enzyme activities remain subject to exogenous control. If the instances so far reported (see Table 1) are quantitatively less extensive than in the whole animal (see *10*), this may reflect the relatively limited number of cell types so far cultured, the profound changes resulting from their adaptation to the conditions of in vitro growth, and the absence of intercellular effects perhaps essential to some of the control mechanisms.

Table 1. Controls of enzyme activity in cultured animal cells.

Substrate induction	End-product repression of enzyme synthesis	Feedback inhibition of enzyme activity	Hormonal induction and repression	Miscellaneous
Arginase (*15, 97*)	Argininosuccinate synthetase (*98*)	Glycine synthesis from glucose (*42*)	Phosphatase (*99, 100*)	Cystine repression of phosphatase (*101*)
Phosphatase (*100, 103*)	Argininosuccinase (*98*)	Proline synthesis from glutamine (*42*)	Tyrosine transaminase (*10*)	Glucose induction of glycogen phosphorylase activity (*103*)
	Aspartic transcarbamylase (*104*)	Purine synthesis (*105*)		DPN inhibition of DPN-ase (*106*)
	β-Glucuronidase (*107*)	Pyrimidine synthesis (*108*)		Induction of virus-uncoating enzyme by pox virus (*58*)
	Glutamine synthetase (*16, 17, 25*)	Serine-phosphate phosphatase (*109*)		Enzyme patterns modified by type of serum in medium (*112*)
	Proline synthesis (*110*)			Induction of δ-aminolevulinic acid synthetase (*112a*)
	Purine synthesis (*111*)			
	Serine synthesis (*109*)			

Although in a number of instances an end-product has been found either to repress the synthesis of the enzymes involved in its formation or to inhibit their activity (see Table 1), such end-product regulation may not be a regularly operative control mechanism for cultured mammalian cells (*11*). Prolonged growth of a variety of human cells in the presence or absence of alanine or proline was found to have little or no effect on the subsequent rate of synthesis from appropriate labeled precursors; and similar growth in glycine inhibited its subsequent synthesis from glucose but not from serine (Table 2). Further, the presence of a pre-formed amino acid usually had no effect on its continuing synthesis, while the provision of alanine and proline actually *increased* the rate at which they

Table 2. End-product control of amino acid synthesis in cultured human cells*. [After Eagle *et al.* (*11*)]

Amino acid synthesized	Isotopically labeled precursor	Effect† of prior "repression" or "de-repression"‡ on rate of synthesis	Effect† of preformed amino acid § on rate of synthesis
Alanine	Glucose	0	>
Glycine	Glucose	<	<
	Serine	0	±
Ornithine	Arginine		0 (in 3 of 4 cell lines tested)
Proline	Arginine	0	0 to >
Proline	Glutamine	0	<
Proline	Ornithine	0	0
Serine	Glucose	±	±
Cystathionine	Methionine		>
Cystine	Methionine		>
Homocystine	Methionine		>

* A variety of diploid and heteroploid cells, both embryonic and adult, and deriving from a number of tissues, including cancer. † 0 = no effect; ± = variable results with different cell lines, or in individual experiments with the same cell line; < = significant repression or feedback inhibition; > = twofold to fourfold increase in amino acid synthesis of end product. ‡ Growth for two to ten generations in a medium containing either none of the end-product amino acid ("de-repression") or exogenous amino acid at concentration of 1 to 10 m*M* ("repression"). § At concentration of 2 to 10 m*M* in medium during labeling experiment.

were synthesized from glucose and ornithine, respectively. In a few instances the presence of pre-formed amino acid inhibited continuing synthesis (for example, of glycine from glucose), but this occurred only when the pre-formed amino acid was present at high and nonphysiological levels (Table 2).

The apparent insensitivity of mammalian cells to end-product control of amino acid synthesis suggests that other and more responsive mechanisms may be involved in the exquisitely controlled homeostasis of the living animal (*12*). The possibility remains, however, that in adapting to the conditions of in vitro cultivation cells lose their capacity to respond to growth control mechanisms which are operative in vivo, perhaps including end-product control of enzyme synthesis and activity. It is possible, also, that the cells which are responsive to such control have not yet been cultured.

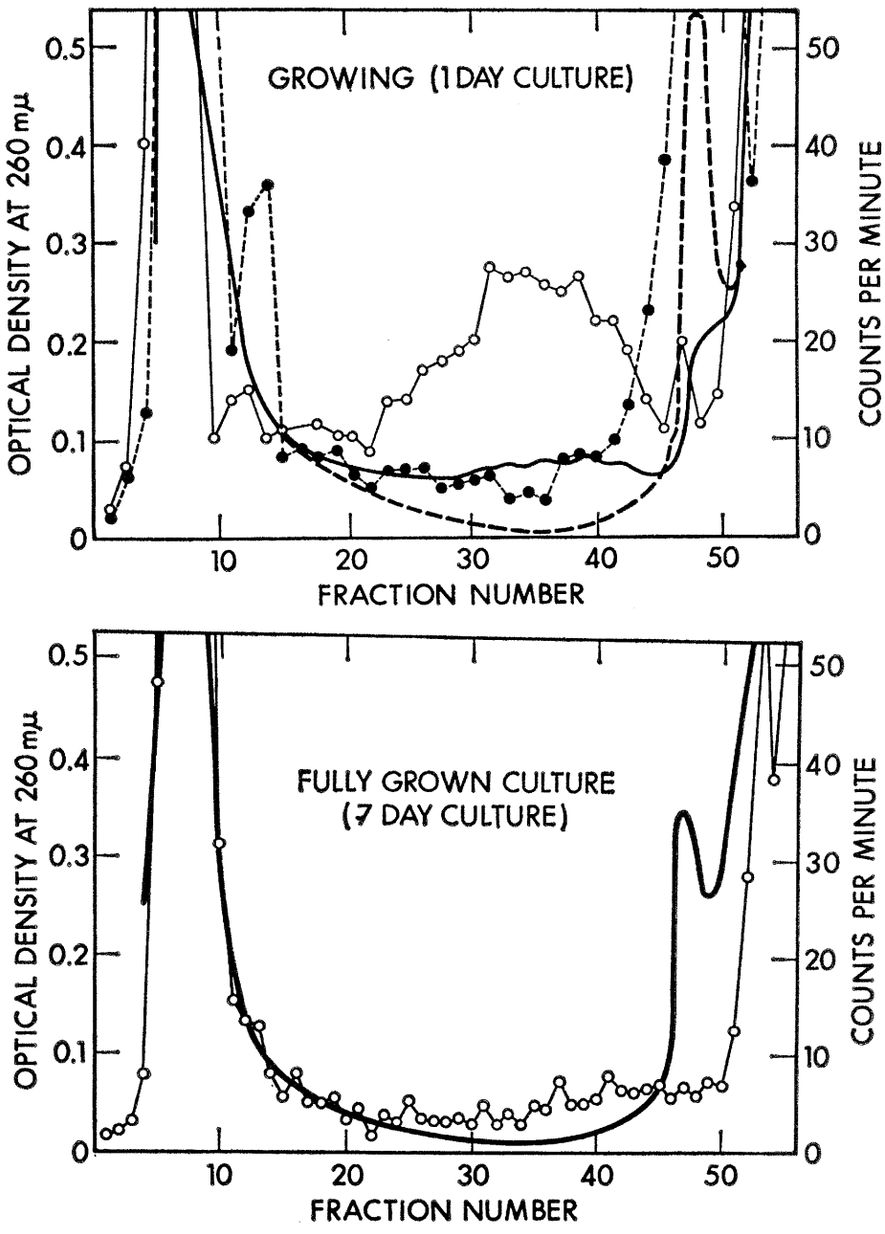

Fig. 1. The disappearance of free cytoplasmic polyribosomes in fully contacted cultures of human diploid fibroblasts. [Sucrose gradient centrifugation of young (1-day) and old (7-day) cultures of diploid fibroblasts (Detroit 510) pulse-labeled for 1 minute with C^{14}-labeled algal hydrolysate.] The washed and trypsinized cells were homogenized in hypotonic buffer, and the cytoplasmic fraction was separated from the nuclei by low-speed centrifugation. Half the cytoplasmic extract was treated with ribonuclease (10 μg). The two cytoplasmic fractions were layered onto sucrose density gradients (2.5 ml of 50-percent sucrose overlaid with 26 ml of 15- to 30-percent sucrose), which were centrifuged (54,000*g* for 2 hours), fractionated, and analyzed for absorbancy at 260 mμ and for C^{14}-amino acid incorporation into material precipitable by trichloroacetic acid. (———) Absorbancy patterns at 260 mμ of untreated extracts; (— —) absorbancy patterns at 260 mμ of extracts treated with ribonuclease; (—o—) pattern of isotope incorporation in untreated extracts; (– –●– –) pattern of isotope incorporation in ribonuclease-treated extracts. The profiles for the fully grown cultures did not change significantly after treatment with nuclease and, for the sake of clarity, these curves are omitted. Fractions 20 to 45 correspond to the polyribosome area, and the adjoining peak (fractions 45 to 50) corresponds to the single ribosomes. [After Levine *et al.* (*28*)]

The continuing active turnover of most of the cell protein (*13*) provides an opportunity for changes in relative enzyme activity. Thus, the presence of specific enzyme-stabilizing compounds which do not affect continuing synthesis may result in an increase in the cellular content of a given enzyme (*14*); Schimke (*15*) has suggested that this may underlie the enhanced arginase activity effected by Mn^{++}. An unexplained increase in the glucose-6-phosphate dehyrogenase (*16, 17*) and glucuronidase (*16*) activities of cultured human cells has been noted as the population density approaches saturation, and cyclic changes in lactic dehydrogenase activity of human cell cultures have also been reported (*17*).

Cellular Interactions

Cellular interaction represents a third type of metabolic control, operative in the whole animal as well as in culture, and involving both similar and dissimilar cells.

Population-dependent growth requirements. A number of compounds that are synthesized by cells must nevertheless be provided in the medium to assure survival and growth. In their absence the cells die because the specific metabolite (or an intermediate in its synthesis) is lost to the medium in amounts which exceed the biosynthetic capacity of the cell. When there are enough cells, however, the medium can be "conditioned"—that is, the concentration in the medium and within the cell can be brought up to metabolically effective levels before the cells die. These paradoxical growth requirements are therefore population-dependent, disappearing at sufficiently high population densities.

The specific instances summarized in Table 3 vary widely with respect to the nature of the metabolite lost to the medium and the critical population den-

sity at which the growth requirement disappears because of mutual feeding of the cells.

1) *End-product leakage.* End-product leakage is illustrated by serine, which is rigorously required for the efficient cloning of cells at population densities of less than 10 to 100 per milliliter (*18*). At that critical population density one finds that the cells can build up the concentrations of serine and of the derived glycine in the medium to the levels necessary to assure the growth of smaller inocula (*19*).

2) *Biosynthetic intermediates.* Biosynthetic intermediates may also be the critical requirement, as illustrated in the case of cystine. In a cystine-free medium, the critical population density which permitted survival and growth of a number of human heteroploid strains varied from 200,000 to 500,000 per milliliter when the cells were given only methionine and glucose [and were then under the necessity of synthesizing and retaining metabolically effective levels of homocystine (or homocysteine), serine, cystathionine, and cystine (or cysteine)] to as few as 10 cells per milliliter when the cells were provided with pre-formed cystathionine and needed to synthesize and retain only cystine (or cysteine) (*19, 20*).

3) *Cofactors.* In primary culture, monkey kidney cells are blocked in the synthesis of glycine from serine (*21*); the requirement for added glycine, however, disappears at extremely high population densities (*19, 22, 23*). The population-dependent requirement here is not for the end product itself but for an essential cofactor, a reduced form of folic acid, which these cells can apparently make to only a limited degree; and the glycine requirement disappears if the cells are provided with folinic acid (*19*).

4) *Glutamine and glutamic acid.* Glutamine, otherwise an essential amino acid, can be replaced by high concentrations of glutamic acid (*24*), in consequence of a tenfold increase in glutamine synthetase activity induced by the glutamic acid (*16, 17, 25*). In such "de-repressed" cells, however, added glutamine is still required at low population densities, presumably because of loss of glutamine from the cells to the medium (*19*). It has recently been shown that glutamic acid is also dispensable (*23*). In cells containing sufficient glutamine synthetase, and therefore not blocked in the synthesis of glutamine from glutamic acid, both amino acids become nonessential for survival and growth at population densities approaching that of a saturated culture. The carbon skeleton then derives from glucose; the source of the α-amino group is not known.

Table 3. Population-dependent nutritional requirements of cultured mammalian cells. Critical population density (cols. 1 and 2) is the density in excess of which the indicated metabolite is no longer required.

Critical population density		Metabolite	Cell strain	Reference
(cells/ml)	(cells/cm²)			
10^6	2×10^5	Cystine	Human heteroploid*	(*19, 20*)
		Glutamic acid or glutamine	All†	(*23*)
		Inositol	HeLa	(*19*)
		Aspartic acid or asparagine	Rat (MDAB tumor)	(*42*)
10^5	2×10^4	Glycine	Monkey kidney	(*21, 23*)
		Serine	Rabbit fibroblast	(*19*)
		Glutamine	All‡	(*24*)
10^4	2×10^3	Asparagine	Rat (MDAB)§	(*42*)
		Asparagine	Rat (Jensen sarcoma)§	
		Pyruvate	Mouse (P388)	(*19, 22*)
$10^3\pm$	$200\pm$	Pyruvate	Diploid cells in general	
10^2	20	Cystine	Human heteroploid ‖	(*19, 20*)
		Pyruvate	Detroit (98–C6B)	(*112b*)
		Serine	Human heteroploid	(*18, 19*)

* Methionine and glucose added to medium at beginning of cell culture. † Provided the cell has adequate amounts of glutamine synthetase, either naturally (as in monkey kidney in primary culture) or after induction with glutamic acid. ‡ Given 20 m*M* glutamic acid as precursor. § Given 20 m*M* aspartic acid as precursor. ‖ Given homocystine and serine as precursors.

5) *Variant cell types.* A number of variant cell types with supposedly specific and unique nutritional requirements have been described (see Table 3). In all but one of these cell types so far examined (the asparagine-dependent Walker 256 carcinoma), these specific requirements also have proved to be population-dependent, a finding which presumably reflects a limited capacity on the part of the cell to synthesize the metabolite which it requires.

6) *Population density and virus synthesis.* The nutritional factors which must be present in the medium in order to permit viral synthesis also depend on the cell population density (*26*).

7) *Differentiation and specialized function.* The clear indications that population density and the associated conditioning of the environment may play an important role both in embryonic differentiation and in the expression of the differentiated function are discussed in following sections.

Culture growth, contact inhibition, and macromolecular synthesis. There is another type of cellular interaction which may also have important physiological implications. Abercrombie and others (*27*) have described the phenomenon of contact inhibition: when diploid cells growing on a glass surface touch, the normal active ruffling movements of a peripheral cellular membrane are inhibited at the point of contact; this leads to the eventual formation of a "monolayer" of cells. As initially discrete cells multiply, and as an increasing proportion of the cell surface is in contact with other cells, there is a pronounced decrease in the rate of protein, RNA, and DNA synthesis (*28*) and in the rate of cellular growth (see *27*). Simultaneously, the free cytoplasmic polyribosomes present in growing cells are no longer demonstrable (Fig. 1). All these effects are reversed within 24 hours after the cultures have been subdivided (*28*). Which of these multiple metabolic changes in the course of culture growth is cause, which is effect, and which are merely associated phenomena not causally related remains to be determined. For the present it is a reasonable working hypothesis that the cellular contact itself sets off a series of reactions, beginning in the surface membrane and ending with a decreased rate of macromolecular synthesis. The elimination or reduction of contact inhibition after spontaneous or virus-induced "transformation" is discussed in a later section.

Even with heteroploid cells which do not exhibit contact inhibition of motility and growth, and which in consequence form heavy multilayered

Table 4. The contrasting properties of cultured diploid and heteroploid cells deriving from human tissues. [After Hayflick and Moorehead (*47*) and Eagle *et al.* (*46*)]

Cells	Cell shape	Growth in suspension	Contact inhibition of movement and perhaps of macromolecular synthesis*	Maximum population density (per cm² of glass surface)	Life expectancy (cell generations)†	Generation time (days)	Cystine (or cysteine) synthesis‡			Alkaline phosphatase §
							From methionine and glucose	From homocystine and serine	From cystathionine	
Diploid ‖	Fibroblastic	0	+	60,000±	20–100	1–14	0	0	0 to ±	Greatly increased in heteroploid as compared with primary cultures; decreased activity of selected variants associated with chromosomal deletions
Heteroploid ¶	Epithelioid	+	0	300,000±	"Immortal"	2/3–1	+	+ +	+ + +	

* See *27* and *28*. † See *46-48*. ‡ See *20* and *46*, and Table 2. § See *43*. ‖ Includes skin cultures from children (BAL, Detroit 510) and adults (F1) and cultures of embryonic adrenal, bone and thymus (*46*). ¶ Includes cell lines deriving from normal tissues (liver, conjunctiva, intestine) and cancer cells (HeLa, KB).

sheets on a glass surface, there is an upper limit to cell population density, in both suspension and surface culture, which is not determined by the exhaustion of the medium. In such cultures, in which the cessation of growth is not related to contact inhibition, there is also a decrease in free cytoplasmic polyribosomes, and an increase in the membrane-associated ribosomes has also been reported (*29*). Although growth inhibitors have been found to develop in such heavy cultures (see, for example, *3*), their nature and their relevance to growth control in vivo, or in vitro, are as yet unknown.

Cellular association. Mixed cell types freshly isolated from tissues frequently reassociate into homotypic aggregates, which may then go on to develop morphologic or biochemical properties characteristic of the parent organ (*30*). This capacity for mutual recognition and association, which obviously depends on the nature of the cellular surface, is often lost in the course of cellular proliferation in vitro (*31, 32*).

Heterotypic cellular interactions. In the living animal the interaction between dissimilar cells, whether in the same or in different organs, is probably more important than that between cells of like type. Only a few systems have been studied in culture which may be considered models for this type of cellular interaction. The differentiation of explants of embryonic-pancreatic (*33*) or kidney (*34, 35*) rudiments is promoted by the linked culture of another bit of tissue in such manner that metabolic products of the inducer tissue stimulate and accelerate the differentiation of the embryonic explant. There have been a number of reports on other, similar tissue interactions in differentiation (*36, 37*). In view of its importance, exploration of this type of interaction between different cell types in serial culture has been surprisingly limited. Medium from a pituitary tumor cell culture, when added to a similar culture of an adrenal tumor, has been found to stimulate the formation of adrenal hormone (*38*). Although a number of growth-inhibitory effects when cells are grown in mixed culture have been reported (*39*), the nature of the interaction and its relevance to growth controls in vivo remain to be determined.

In sum, cellular interactions profoundly modify the metabolic activity of cultured cells and are evidenced in terms of nutritional requirements, growth rates, the expression of specialized functions, and morphogenesis. Only a beginning has been made in the elucidation of these effects.

Genetic Control

Cultured cells from patients with inborn errors of metabolism. Skin cultures from patients with genetically determined metabolic errors sometimes exhibit the biochemical defect (*40*) and are potentially useful tools for the exploration of the biochemical deficiency, as well as for the study of genetic transformation, recombination, and, ultimately perhaps, mapping. Usually, however, the metabolic error involves an enzyme or enzymes not normally present in the skin, hence the defect is not expressed in the cultured cells.

Variant cells. A number of cell lines isolated from normal animals but with unusual nutritional requirements or metabolic activities have been described. Many of these requirements have proved to reflect a lower-than-normal capacity to synthesize the metabolite in question, rather than an absolute block, and these requirements disappear at high population densities (see Table 2). Since some of these requirements are evidenced in the primary culture, immediately after explantation, they presumably reflect the varying phenotypic expression of a normal genome, rather than somatic mutations developing in culture.

The broad similarities that have been found in the nutritional requirements and the metabolic activities of cultured cells of various types may reflect only the limited number of cell lines so far cultivated, and interesting metabolic diversities may appear only with the development of techniques for the successful cultivation of conceivably more exacting cell types. It is possible, also, that the metabolic uniformity of cultured mammalian cells reflect the basic uniformity of the biochemical processes involved in cellular replication, whereas the expression of organ-specific function, involving as it does unique enzymic activities, may also involve the provision of unique metabolites.

"Spontaneous" chromosomal aberrations and cell metabolism. Many of the human cell lines currently available for experimental study are heteroploid. While those deriving from malignant tissue (for example, HeLa and KB) may have had an abnormal karyotype at the time of original isolation, those deriving from normal tissue have become aneuploid in the course of their serial propagation. Some of the heteroploid "transformants" may reflect errors, such as mistaken labeling or the

contamination of one cell line by another which grew out selectively. There are a number of documented instances of the latter occurrence (see *7*), and an even larger number of cases have probably not been reported.

The pronounced differences between the properties of heteroploid and diploid cultured human cells are illustrated in Table 4. These include differences in morphology, susceptibility to contact inhibition, maximum population density, life expectancy, and enzymatic activities. It is tempting to ascribe all these differences to the developing heteroploidy itself—for example, to gene dosage effects which occasionally, but not invariably, endow the aneuploid cell with a selective advantage. This is not to say that the development of a chromosomal abnormality regularly has the same phenotypic effect in cultured human cells, and that every heteroploid human cell has all the properties listed in Table 4. The association is statistical but not invariable. Thus, some diploid human cells are epithelioid (*41*), and not all heteroploid human cells are derepressed with respect to cystine synthesis (*42*). A possible causal relationship between karyotypic and phenotypic changes has been suggested in relation to alkaline phosphatase activity (*43*) and a number of other properties, including resistance to aminopterin and viruses (*44*). It should be emphasized that, unlike karyotypic changes in mouse cells, and contrary to statements in the literature, karyotypic changes in cultured human cells do not necessarily signify a malignant transformation as judged by homo- or heterotransplantability (*45*).

However attractive the working hypothesis that the developing heteroploidy is responsible for the phenotypic changes summarized in Table 4, that hypothesis must be regarded as tentative in the absence of matched pairs of diploid human cells and heteroploid strains deriving from them with which they can be compared. For despite the widespread impression that human cells in culture regularly become aneuploid and malignant, "it is a curious and puzzling fact that most of the heteroploid human cultures now available which originated from normal tissues were isolated prior to 1960, that almost all are epithelioid, and have continued to divide with a generation time of 16 to 24 hours for more than 5 years. Since 1960, however, with the possible exception of certain amnion cultures, most of the human strains isolated from normal tissue have been fibroblastic, have died out after 20 to 100 cell generations in vitro, and have remained diploid until the terminal stages of their life span" (*46*). Those terminal chromosomal aberrations only rarely eventuate in permanent culture strains (*47–49*).

In contrast to human cells, mouse cell cultures regularly "transform" in culture to form permanent lines. After a varying number of culture generations, during which the growth rate declines markedly, islands of cells appear which grow and eventually become grossly heteroploid (*50, 51*). Many of the differences between diploid and heteroploid human cells listed in Table 4 are evidenced in these mouse cultures as well; but, in addition, those heteroploid cells usually form cancers on inoculation into the appropriate host (*51, 52*). Similar "transformations" in the course of serial propagation have been noted in other species (*53, 54*), but these karyotypic changes are not necessarily associated with profound phenotypic alterations (*54*).

Hybridization. The successful hybridization of cultured mouse cells of varying karyotype, and with associated phenotypic differences, provides a potentially powerful tool for the genetic analysis of cultured cells and for analysis of the metabolic consequences of changes in the chromosomal complement (*55*).

Somatic mutation and transformation by DNA. By appropriate selection a number of drug-resistant variants of cultured cells have been obtained, and similar variants with special protein requirements or variants resistant to viruses have been reported (see *7* for a partial summary). From analogy to antibiotic-resistant bacteria, it has been proposed that the drug-resistant variants reflect somatic mutation at a specific gene locus (*56*), and the transformation of normal cells by DNA of a resistant variant has in fact been reported (*57*).

Metabolic Effects of Viral Infection

The profound metabolic effects of viral infection are obviously relevant to a discussion of metabolic controls. Cytolytic viruses such as poliovirus or vaccinia turn off most of the cell's own biosynthetic processes and take over its protein-synthesizing machinery by virtue of a virus-specific messenger RNA, the production of which is encoded in the genome of the infecting virus. The synthesis of viral nucleic acid and protein continues long enough for the production of complete virus, after which the cell dies.

Recent work in this laboratory (*58*) makes it clear that the metabolic effects of the infecting virus do not, however, depend solely on information encoded in the viral nucleic acid. One of the earliest events after the penetration of vaccinia virus into the host cell is the release from the viral coat of a compound which apparently induces the synthesis of a host protein, presumably an enzyme, which strips the virus of its protein coat to liberate the infective viral DNA. The interferons which are produced by some virus-infected cells similarly appear to be host proteins, the production of which is induced by viral infection but encoded in the host genome (*59*).

Even a cursory survey of the extensive and rapidly growing literature on the biochemical events associated with viral synthesis is quite beyond the scope of this article. It is, however, evident that the elucidation of a process which involves the complete inhibition of the host cell's own biosynthetic processes, the induction of host enzymes normally repressed, and the subversion of the biosynthetic machinery of the cell to the production of viral protein and viral nucleic acid cannot help but throw light on the normally operative metabolic controls. In the latter two respects, study of the oncogenic ("transforming") viruses should prove even more instructive. The cell is not killed, but is profoundly modified by the viral infection, which produces a number of heritable changes (*60*). While some of these [for example, the appearance of a new antigen (*61*)] are quite specific, in other respects (release of contact inhibition, greater maximum population density, morphologic and karyotypic changes, immortality of the transformed cell, ability to form a cancer in an appropriate host) the virus-transformed cells closely resemble the aneuploid cells which may appear spontaneously in culture. As with the latter, not every virus-transformed cell acquires all these properties. Not all virus-transformed cells will grow indefinitely in culture (*62*), and not all have been shown to be malignant (*54*). Further, the proposed causal relationship between the developing aneuploidy and the malignant transformation has been questioned by several workers (*63*). The possibility must be considered, also, that at least some of the phenotypic

changes in virus-transformed cells are not encoded in the viral genome. Some of them may reflect induction of the host cell by viral constituents (*58, 59*), and others may reflect chromosomal aberrations which are merely initiated by viral infection, and which in their early stages are perhaps not discernible (*64*). There is, however, the converse possibility: that the "spontaneous" transformations regularly observed in mouse cell cultures, and occasionally in human cells as well, may be due to unrecognized viral infection.

Organ-Specific Functions in Cultured Cells

In general, and with the exceptions noted below, most dispersed cells in serial culture do not carry out the functions of the tissues from which they were derived (see *7*). For some organs and for some cell types this probably means only that the cells which grow out are not the specific functioning cells (*7*, p. 626; *65*) and that the latter either (i) cannot multiply under any condition, (ii) are overgrown by other cell types with a selective advantage in vitro, or (iii) have unique nutritional requirements over and above the requirement for the 28 factors which suffice for the growth of most animal cells (*2*). The possibility of specific nutritional requirements is clearly illustrated by the effects of specific growth factors on the in vitro multiplication of neural cells (*66*) and skin epidermis (*67*), and by the stimulation of an adrenal tumor culture by ascorbic acid (*30*).

Even if the correct cell type is cultured, the in vitro conditions may not be conducive to the expression of a specialized function.

1) *Loss of enzymes.* An early decrease in many enzymatic activities has been noted when cells wander out from culture explants or are grown from trypsinized suspensions (*8*), and further progressive decrease may occur in the course of their serial propagation. This diminution in enzyme activity frequently involves enzymes crucial to the specialized function (see *7*, p. 625). Even when specific enzymes have not been implicated, most cell lines lose their organ-specific functions in the course of proliferation in vitro (*7, 32, 68*), as well as their organ-specific antigens (*9*) and their capacity for self-recognition and re-aggregation (*32, 69*).

2) *Population density.* The population density of even saturated cultures is low relative to that of the intact organ, favoring the loss of intermediates, cofactors, or even macromolecules essential to function. Several instances have in fact been reported of specialized functions retained in cultured cells but expressed only at high population densities—for example, melanin production by iris and retinal epithelium (*70, 71*), hormone production by adrenal and pituitary tumor cultures (*30*), collagen formation in cultures of fibroblasts (*72*), cartilage production by chondrocytes (*73*), and myofibril formation by myoblasts (*74*). The marked cyclic changes in the specific activity of certain enzymes in cultured human cells (*16, 17*) is of particular relevance to this phenomenon.

3) *Growth versus function.* This dependence of specialized function on cell density may, however, involve factors other than cell leakage. The thesis has often been stated that a cell cannot simultaneously grow and carry out specialized function—for example, that the rapid replication of DNA and the synthesis of structural components of the cell may preclude the continuing formation of the specific messenger RNA necessary for the maintenance of specialized function. The profound inhibitory effects of cellular contact on macromolecular synthesis may well be a related phenomenon. The antithesis between growth and function is, however, apparently not valid as a generalization, for some growing cells do carry out specialized functions (*7, 74, 75*).

4) *Specific requirements for function.* The expression of specialized function is further complicated by the frequent requirement for compounds not necessary for growth. The effect of vitamin A (*76*) and of specific growth-stimulating protein (*67*) on the keratinization of epidermis, of vitamin C on collagen formation by mouse fibroblasts (*72*), and of hormones on the formation of collagen (*77*), melanin (*78*), antibodies (*79*), and mucopolysaccharides (*80*) are cases in point. Even when the inducer or cofactor is known, it may be so toxic that it would have to be continuously introduced into the medium at the low and presumably nontoxic levels required for function (see *81*). Further, the possibility cannot be excluded that, in organized tissue, other cells immediately adjacent to the specific functioning cell transmit compounds necessary for function by cell-to-cell contact.

Given all these difficulties, it is both surprising and encouraging to note that a number of cell lines have been obtained which continue to carry out a specialized function after many generations in dispersed culture (see Table 5). An even larger number of cell types which "de-differentiate" on serial cultivation retain their organ-specific function in primary culture for days or even weeks (*7*, p. 124; *68*; *82*), and often when a specific functioning cell cannot be cultured, fragments of tissue retain an organ-specific function for appreciable periods in vitro (see, for example, *7*, p. 123; *83*). It is therefore now possible to delimit, in a broad spectrum of cell types, the conditions necessary for the expression of a differentiated organ-specific function. These findings should have an important application in the study of embryonic differentiation.

Prospects

A question was posed at the outset as to the relevance and applicability of experimental findings with discrete cultured cells to such complex phenomena as embryonic differentiation, senescence, and cancer. The progress of the past 5 years justifies an optimistic view—not that these and similar processes can possibly be understood in full from the study of cultured cells or from tissue explants, but that such cultures provide reproducible and simple experimental systems which can yield data of significance, and possibly of key significance, to an understanding of fundamentally important biological processes.

Differentiation. The fact that a number of cell types have now been isolated which continue to carry out an organ-specific function in culture provides an opportunity to delimit the environmental conditions necessary for the maintenance of that function. Only when these conditions have been defined for each cell type can one reasonably hope to explore in culture the complex process by which the specific cell type had acquired that differentiated function in vivo. There are in fact striking parallelisms between the conditions necessary for the development of specialized function and those necessary for its expression. Just as population density may profoundly affect the expression of function by a differentiated cell, in the differentiation of embryonic rudiments also, there may be a critical mass below which differentiation does not occur (*84*); and, in

vitro, dissociated chondrocytes do not differentiate into cartilage unless they are compacted into a pellet (*32, 68, 69*). Whether the crucial factor here is population density and cell leakage or an intimate association which permits an as yet undefined cellular interaction (see *85*) remains to be determined. Further, just as cells of different type must sometimes interact in order to permit the expression of a differentiated function in vitro, interaction between heterotypic tissues has been shown to be crucial for the differentiation of pancreatic (*33*), kidney (*34, 35*), thymus (*35*), and cartilage (*36*) rudiments as well as epidermis (*37*; see also *4*). The nature of that interaction has been discussed by Grobstein (*86*). Finally, hormonal and vitamin effects are known to be involved in some instances of both differentiation (*76*) and function.

With the rapidly evolving insights into the mechanisms and controls of protein synthesis, and the development of new techniques for isolating and characterizing both the macromolecules involved and the organelles with which they are associated, the time is perhaps ripe for exploration of differentiation and morphogenesis at the cellular level. In such studies, both explant cultures and dispersed cell cultures will find important and mutually supplementary application.

Cancer. Cultured cells may undergo profound changes in the course of their serial rapid propagation (see Table 4) (*87, 88*). In particular, the cells may become malignant—that is, escape in part from the control mechanisms which normally limit cellular proliferation in vivo, so that, upon inoculation into the appropriate host and in the right site, the altered cells may grow to form an invasive tumor with the histopathologic features of cancerous tissue. Not all the spontaneous "transformants," recognized by changes in karyotype, growth pattern, or metabolic activity, are, however, malignant, as judged by their homo- or heterotransplantability (*45*); and some morphologically "normal" cells are malignant (*89*). Further, on prolonged cultivation in vitro, a number of initially malignant cells have become relatively nonmalignant, with no gross karyotypic changes (*90*). The transformation of cells by oncogenic viruses is accompanied by many of the phenotypic changes noted in spontaneous transformants, but, here again, the changes are not necessarily all associated.

Table 5. Serially cultured cells which retain organ-specific functions.

Organ-specific product or function	Cell type	Reference
Adrenal hormone	Adrenal tumor	(*38*)
Collagen	Mouse and human fibroblasts	(*7*, p. 623; *72*; *77*)
Histamine	Mast cell tumor	(*113*)
Melanin	Chick iris and retina	(*71*)
Melanin	Mouse melanoblasts	(*70*)
Mucopolysaccharides	Rat eye connective tissue	(*75*)
Mucopolysaccharides	Rous sarcoma	(*115*)
Mucopolysaccharides	Mouse fibroblasts	(*114*)
Myofibril formation	Myoblasts	(*74*)
Nerve growth factor	Neuroblastoma	(*116*)
Pituitary hormone	Pituitary tumor	(*38*)
Pituitary hormone	Normal pituitary*	(*117*)
Serotonin	Mast cell tumor	(*113*)
Spermatocytogenesis	Guinea pig testicular cells	(*118*)

* Negative results in primary cultures of pituitary tissue have, however, been reported (*119*).

The foregoing set of observations merely illustrates the innumerable observations concerning normal and malignant cells which require sorting out. Is a chromosomal aberration (or a point mutation) the primary event responsible for the malignant character of some spontaneous transformants, as has been often suggested [see recent summaries by Stich (*91*) and Harris (*87*)]? In that case, does the "transformed" cell now synthesize compounds which permit it to escape from normal control mechanisms, or does it fail to make compounds the accumulation of which normally turns off cellular replication? Is there a general loss or derangement of normal controls of enzyme synthesis (see *92*)? Does the escape from contact inhibition shown by many aneuploid cells reflect the loss of a recognition signal in consequence of which the transformed cell in vivo also grows far beyond normal limits? Is viral transformation analogous to spontaneous transformation, the information for the phenotypic changes deriving, however, from the viral genome rather than a somatic mutation? Or does the virus act indirectly, by initiating progressive changes in the host genome?

These and similarly obvious questions have been posed repeatedly in the past, and one can lengthen the list at will. The relevant point is that these questions in part grow out of studies with cultured cells, and that some of the answers can be sought in the same system.

Senescence. The rate of growth of most cultured human diploid fibroblasts becomes progressively slower in the course of their serial propagation, and eventually they die (unless they have in the meantime become transformed). This is clearly an aging process at the cellular level, the elucidation of which merits exploration even if it should prove to bear no relationship to the senescence of the whole animal.

Cytotoxic agents. The mechanism of action of a number of compounds which profoundly modify cellular metabolism has been studied in some detail. Thus, the findings that actinomycin D combines with DNA to prevent the synthesis of messenger RNA (*93*) and that puromycin combines with the growing polypeptide chain to stop protein synthesis (*94*) have provided powerful tools for a wide variety of studies at the cellular level. Over the past decade, in particular, an intensive search for antitumor agents has uncovered thousands of highly cytotoxic compounds (*1, 95*). It is a reasonable surmise that the exploration of their mode of action could similarly yield a rich harvest of new and important information on metabolic controls (see *96*).

Summary

A number of apparently unrelated factors are known to have a profound effect on the metabolism of cultured mammalian cells; and some of these may be operative as metabolic controls in the whole animal as well. The more complete exploration of (i) homotypic and heterotypic cellular interactions, (ii) the spontaneous transformations sometimes observed in cultured cells, (iii) the mode of action of cytotoxic agents, (iv) the multiple metabolic effects of viral infection, and (v) the conditions necessary for the maintenance of specialized function in cultured cells, can be expected to throw light on the basic mechanisms underlying such complex processes as differentiation, senescence, and cancer.

References and Notes

1. In a recent review on cell culture and chemotherapy [G. E. Foley and S. S. Epstein, in *Advances in Chemotherapy* (Academic Press, New York, 1964), vol. 1] more than 500 references dealt with one limited topic, the use of cultured cells for the assay of cytotoxicity and the screening of candidate antitumor agents.
2. H. Eagle, *Science* **122**, 501 (1955); **130**, 432 (1959).
3. E. P. Cohen and H. Eagle, *J. Exptl. Med.* **113**, 467 (1961).
4. H. Eagle, K. A. Piez, M. Levy, *J. Biol. Chem.* **236**, 2039 (1961).
5. K. A. Piez and H. Eagle, *ibid.* **231**, 533 (1958); H. Eagle and K. A. Piez, in *Amino Acid Pools* (Elsevier, Amsterdam, 1962), p. 694.
6. H. Eagle, *Proc. Natl. Acad. Sci. U.S.* **46**, 427 (1960).
7. L. Levintow and H. Eagle, *Ann. Rev. Biochem.* **30**, 605 (1961).
8. H. Burlington, *Am. J. Physiol.* **197**, 68 (1959); A. Fujimoto and I. Harary, *Biochim. Biophys. Acta* **86**, 74 (1964); B. Holmberg, *Cancer Res.* **21**, 1386 (1961); M. Jones, R. M. Featherstone, S. L. Bonting, *J. Pharmacol. Exptl. Therap.* **116**, 114 (1956); H. Kuramitsu and I. Harary, *Biochim. Biophys. Acta* **86**, 65 (1964); I. Lieberman and P. Ove, *J. Biol. Chem.* **233**, 634 (1958); I. Lieberman, R. Abrams, N. Hunt, P. Ove, *ibid.* **238**, 3955 (1963); J. Philip and E. S. Vesell, *Proc. Soc. Exptl. Biol. Med.* **110**, 582 (1962); G. S. Turner, *Nature* **193**, 164 (1962); R. Wu, *Cancer Res.* **19**, 1217 (1959).
9. E. Weiler, in *Carcinogenesis* (Little, Brown, Boston, 1959), p. 165.
10. H. C. Pitot, C. Peraino, P. A. Morse, Jr., V. R. Potter, in "Metabolic Control Mechanisms in Animal Cells," *Natl. Cancer Inst. Monograph 13* (1964).
11. H. Eagle, C. Washington, M. Levy, G. Barton, "End Product Control of Amino Acid Synthesis in Human Cell Cultures," in preparation.
12. A. Pardee and A. C. Wilson, *Cancer Res.* **23**, 1483 (1963); R. T. Schimke, *J. Biol. Chem.* **238**, 1012 (1963).
13. H. Eagle, K. A. Piez, R. Fleischman, V. I. Oyama, *J. Biol. Chem.* **234**, 592 (1959).
14. T. B. Bojarski and H. H. Hiatt, *Nature* **188**, 1112 (1960); R. T. Schimke, M. B. Brown, E. Smallman, *Ann. N.Y. Acad. Sci.* **102**, 587 (1963).
15. R. T. Schimke, in "Metabolic Control Mechanisms in Animal Cells," *Natl. Cancer Inst. Monograph 13* (1964).
16. R. DeMars, *ibid.*
17. C. DeLuca and H. M. Nitowsky, *Biochim. Biophys. Acta* **89**, 208 (1964).
18. R. Z. Lockart, Jr., and H. Eagle, *Science* **129**, 252 (1959).
19. H. Eagle and K. Piez, *J. Exptl. Med.* **116**, 29 (1962).
20. H. Eagle, K. A. Piez, V. I. Oyama, *J. Biol. Chem.* **236**, 1425 (1961).
21. H. Eagle, A. E. Freeman, M. Levy, *J. Exptl. Med.* **107**, 643 (1958); C. Rappaport and J. L. Melnick, *Federation Proc.* **16**, 429 (1957).
22. H. Eagle, in *The General Physiology of Cell Specialization* (McGraw-Hill, New York, 1963), p. 148.
23. ———, "The Population-Dependent Requirement by Cultured Mammalian Cells for Metabolites Which They Can Synthesize. II, Glycine, Glutamic Acid and Glutamine," in preparation.
24. ———, V. I. Oyama, M. Levy, C. L. Horton, R. Fleischman, *J. Biol. Chem.* **218**, 607 (1956).
25. R. DeMars, *Biochim. Biophys. Acta* **27**, 435 (1958).
26. J. E. Darnell, Jr., and H. Eagle, *Virology* **6**, 556 (1958); H. Eagle and K. Habel, *J. Exptl. Med.* **104**, 271 (1956).
27. M. Abercrombie and E. J. Ambrose, *Exptl. Cell Res.* **15**, 332 (1958); ———, *Cancer Res.* **22**, 525 (1962); M. Abercrombie and J. E. Heaysman, *Exptl. Cell Res.* **6**, 293 (1954).
28. L. Levine, Y. Becker, C. Boone, H. Eagle, "Contact Inhibition, Macromolecular Synthesis and Free Polyribosomes in Cultured Human Cells, *Proc. Natl. Acad. Sci. U.S.*, in press.
29. B. Goldberg and H. Green, *J. Cell Biol.* **22**, 227 (1964); R. Love, G. P. Studzinski, A. M. Clark, E. R. Tressan, paper presented Sept. 1964 at the 11th International Congress of Cell Biology.
30. K. Dabrowska-Piaskowska, *Exptl. Cell Res.* **16**, 315 (1959); J. W. Grover, *Develop. Biol.* **3**, 555 (1961); A. A. Moscona, *Developing Cell Systems and Their Growth* (Proc. 18th Growth Symposium, Society for the Study of Development and Growth); M. S. Steinberg, in *Cellular Membranes in Development* (Academic Press, New York, 1964), p. 321; ———, *Science* **141**, 401 (1963); J. P. Trinkaus, *Ann. N.Y. Acad. Sci.* **100**, 413 (1963); P. Weiss, *Can. Cancer Conf.* **5**, 241 (1963); ——— and A. Moscona, *J. Embryol. Exptl. Morphol.* **6**, 238 (1958).
31. M. Moskowitz, *Nature* **200**, 854 (1963).
32. S. R. Hilfer, *Develop. Biol.* **4**, 1 (1962); H. Holtzer, J. Abbott, J. Lash, S. Holtzer, *Proc. Natl. Acad. Sci. U.S.* **46**, 1533 (1960); Y. Kuroda, *Exptl. Cell Res.* **30**, 446 (1963); ———, *ibid.* **35**, 337 (1964); A. A. Moscona, *J. Cellular Comp. Physiol.* **60**, suppl. 1, 65 (1962); P. Weiss, *ibid.*, p. 45.
33. N. Golosow and C. Grobstein, *Develop. Biol.* **4**, 242 (1962).
34. C. Grobstein, *Exptl. Cell Res.* **10**, 424 (1956); ——— and A. J. Dalton, *J. Exptl. Zool.* **135**, 57 (1957).
35. R. Auerbach, *Develop. Biol.* **2**, 271 (1960).
36. J. W. Lash, in *Cytodifferentiation and Macromolecular Synthesis*, D. Locke, Ed. (Academic Press, New York, 1963), p. 235; G. Strudel, *Develop. Biol.* **4**, 67 (1962).
37. C. B. McLoughlin, *J. Embryol. Exptl. Morphol.* **9**, 370 (1961).
38. V. Buonassissi, G. Sato, A. I. Cohen, *Proc. Natl. Acad. Sci. U.S.* **48**, 1184 (1962); G. H. Sato and V. Buonassissi, in "Metabolic Control Mechanisms in Animal Cells," *Natl. Cancer Inst. Monograph 13* (1964), p. 81.
39. M. Abercrombie, J. E. M. Heaysman, H. M. Karthauser, *Exptl. Cell Res.* **13**, 276 (1957); H. Katsuta and T. Takaoka, *J. Natl. Cancer Inst.* **32**, 963 (1963); F. E. Oldfield, *Exptl. Cell Res.* **30**, 125 (1963); E. Shelton, *J. Natl. Cancer Inst.* **32**, 709 (1964).
40. J. Dancis, V. Jansen, J. Hutzler, M. Levitz, *Biochim. Biophys. Acta* **77**, 523 (1963); S. M. Gartler, E. Gandini, P. Cepellini, *Nature* **193**, 602 (1962); R. S. Krooth, R. R. Howell, H. B. Hamilton, *J. Exptl. Med.* **115**, 313 (196— R. S. Krooth and A. N. Weinberg, *ibid.* **113**, 1155 (1961).
41. R. M. Richart, *Cancer Res.* **24**, 662 (1964); ——— and P. A. Corfman, *Science* **144**, 65 (1964).
42. H. Eagle *et al.*, unpublished observations.
43. L. DeCarli, J. J. Maio, F. Nuzzo, *J. Natl. Cancer Inst.* **31**, 1501 (1963); U. R. Konigsberg and H. M. Nitowsky, *ibid.* **29**, 699 (1962); J. J. Maio and L. L. DeCarli, *Nature* **196**, 600 (1962).
44. M. Vogt, *J. Cellular Comp. Physiol.* **52**, suppl. 1, 271 (1958); *Genetics* **44**, 1257 (1959).
45. G. E. Foley and A. H. Handler, *Exptl. Biol. Med.* **94**, 661 (1957); *Ann. N.Y. Acad. Sci.* **76**, 506 (1958); *Exptl. Cell Res.* **33**, 591 (1964).
46. H. Eagle, C. Washington, S. M. Friedman, M. Levy, G. Barton, "Cystine Synthesis by Diploid and Heteroploid Human Cells," in preparation.
47. L. Hayflick and P. S. Moorehead, *Exptl. Cell Res.* **25**, 585 (1961).
48. C. P. Miles, *Cancer Res.* **24**, 1070 (1964).
49. E. Saksela and P. S. Moorehead, *Proc. Natl. Acad. Sci. U.S.* **50**, 390 (1963).
50. T. C. Hsu, D. Billen, A. Levan, *J. Natl. Cancer Inst.* **27**, 515 (1961); K. H. Rothfels, E. B. Kupelweiser, R. C. Parker, *Can. Cancer Conf.* **8**, 191 (1963); K. H. Rothfels and R. C. Parker, *J. Exptl. Zool.* **142**, 507 (1959).
51. A. Levan and J. J. Biesele, *Ann. N.Y. Acad. Sci.* **71**, 1022 (1958); G. J. Todaro and H. Green, *J. Cell Biol.* **17**, 299 (1963).
52. K. K. Sanford, G. L. Hobbs, W. R. Earle, *Cancer Res.* **15**, 162 (1956); K. K. Sanford, R. M. Merwin, G. L. Hobbs, M. C. Fioramonti, *J. Natl. Cancer Inst.* **21**, 121 (1958).
53. E. A. McCulloch and R. C. Parker, *Can. Cancer Conf.* **2**, 152 (1957); J. C. N. Westwood, I. A. Macpherson, D. H. J. Titmuss, *British J. Exptl. Pathol.* **38**, 138 (1957).
54. D. K. Ford, C. Boguszewski, N. Auersperg, *J. Natl. Cancer Inst.* **26**, 691 (1963).
55. G. Barski and F. Cornefert, *ibid.* **28**, 801 (1962); G. Barski, S. Sorieul, F. Cornefert, *ibid.* **26**, 1269 (1961); V. Defendi, B. Ephrussi, H. Koprowski, *Nature* **203**, 495 (1964); B. Ephrussi and S. Sorieul, *Compt. Rend.* **254**, 181 (1962); ———, *Univ. Mich. Med. Bull.* **28**, 347 (1962); D. Gershon and L. Sachs, *Nature* **198**, 912 (1963); J. W. Littlefield, *Science* **145**, 709 (1964); S. Sorieul and B. Ephrussi, *Nature* **190**, 653 (1961).
56. W. Szybalski, *Exptl. Cell Res.* **18**, 588 (1959); ——— and M. J. Smith, *Proc. Soc. Exptl. Biol. Med.* **101**, 662 (1959).
57. E. H. Szybalska and W. Szybalski, *Proc. Natl. Acad. Sci. U.S.* **48**, 2026 (1962); W. Szybalski, Z. Opara-Kubinsta, Z. Lorkiewicz, E. Ephrati-Elizur, S. Zamenhof, *Nature* **188**, 743 (1960).
58. W. K. Joklik, *J. Mol. Biol.* **8**, 263 (1964); *ibid.*, p. 277.
59. S. Baron, S. Barban, C. E. Buckler, *Science* **145**, 814 (1964); E. Heller, *Virology* **21**, 652 (1963).
60. R. Dulbecco and M. Vogt, *Proc. Natl. Acad. Sci. U.S.* **46**, 1617 (1960); B. Hampar and S. A. Ellison, *Nature* **192**, 145 (1961); D. Medina and L. Sachs, *Virology* **10**, 387 (1960); M. Stoker and I. Macpherson, *ibid.* **14**, 359 (1961); M. Vogt and R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.* **46**, 365 (1960).
61. R. Bases, *Cancer Res.* **24**, 1216 (1964); P. H. Black, W. P. Rowe, H. C. Turner, R. J. Huebner, *Proc. Natl. Acad. Sci. U.S.* **50**, 1148 (1963); K. Habel, *J. Exptl. Med.* **115**, 181 (1962); ———, *Virology* **18**, 553 (1962); J. H. Pope and W. P. Rowe, *J. Exptl. Med.* **120**, 121 (1964).
62. A. W. Moyer, R. Wallace, H. R. Cox, *J. Natl. Cancer Inst.* **33**, 227 (1964); H. M. Shein, J. F. Enders, L. Palmer, E. Grogan, *Proc. Soc. Exptl. Biol. Med.* **115**, 618 (1964).
63. K. E. Hellstrom, I. Hellstrom, H. O. Sjogren, *J. Natl. Cancer Inst.* **30**, 1239 (1963); M. A. Rich, R. Tsuchida, R. Siegler, *Science* **146**, 252 (1964).
64. W. W. Nichols, *Hereditas* **50**, 53 (1963).
65. G. Sato, L. Zaroff, S. E. Mills, *Proc. Natl. Acad. Sci. U.S.* **46**, 963 (1960).
66. R. Levi-Montalcini, *Science* **143**, 105 (1964); ——— and S. Cohen, *Ann. N.Y. Acad. Sci.* **85**, 324 (1960).
67. S. Cohen, in "Metabolic Control Mechanisms in Animal Cells," *Natl. Cancer Inst. Monograph 13* (1964), p. 13.
68. J. P. Cobb and D. G. Walker, *Cancer Res.* **20**, 858 (1960); K. E. Ebner, E. C. Hageman, B. L. Larson, *Exptl. Cell Res.* **25**, 555 (1961); H. Grossfeld, *Biochim. Biophys. Acta* **74**, 193 (1963); I. Harary and B. Farley, *Exptl. Cell Res.* **29**, 451 (1963); I. Harary and E. Sato, *Biochim. Biophys. Acta* **82**, 614 (1964); H. Kuramitsu and I. Harary, *ibid.* **86**, 65 (1964); D. J. Prockop, O. Pettengill, H. Holtzer, *ibid.* **83**, 189 (1964); R. H. Smith and S. F. Jackson, *J. Biophys. Biochem. Cytol.* **3**, 913 (1957); J. M. Twarog and B. L. Larson, *Exptl. Cell. Res.* **28**, 350 (1962).
69. J. W. Grover, *Exptl. Cell Res.* **24**, 171 (1961).
70. M. Foster, *Anat. Record* **125**, 622 (1956); M. Foster, R. Cook, Jr., T. A. Stamas, *J. Exptl. Zool.* **132**, 1 (1956); J. R. Whittaker, *Develop. Biol.* **8**, 99 (1963).
71. B. Ephrussi and H. M. Temin, *Virology* **11**, 547 (1960); L. Doljanski, *Compt. Rend. Soc. Biol.* **105**, 343 (1930–31).
72. B. Goldberg and H. Green, *J. Cell Biol.* **22**, 227 (1964); B. Goldberg, H. Green, G. J. Todaro, *Exptl. Cell Res.* **31**, 444 (1963); H. Green and B. Goldberg, *Nature* **200**, 1097 (1963).
73. T. S. Okada, *Exptl. Cell Res.* **16**, 437 (1959); F. E. Stockdale, J. Abbott, S. Holtzer, H. Holtzer, *Develop. Biol.* **7**, 293 (1963).
74. I. R. Konigsberg, *Science* **140**, 1273 (1963).
75. E. H. Davidson, V. G. Allfrey, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.* **49**, 53 (1963).
76. H. B. Fell, *J. Embryol. Exptl. Morphol.* **10**, 389 (1962); U. Laschet, *Z. Vitamin-, Hormon- Fermentforsch.* **12**, 1 (1961); N. K. Wessells, *Exptl. Cell Res.* **24**, 131 (1961).

77. C. W. Castor and K. D. Muirden, *Lab. Invest.* **13**, 560 (1964).
78. B. Kosto, G. E. Pickford, M. Foster, *Endocrinology* **65**, 869 (1959); R. R. Novales, *Anat. Record* **134**, 617 (1959); T. T. Tchen, R. N. Ameraal, K. Kim, C. M. Wilson, W. Chain, F. Hu, in "Metabolic Control Mechanisms in Animal Cells," *Natl. Cancer Inst. Monograph 13* (1964), p. 67.
79. C. T. Ambrose, *J. Exptl. Med.* **119**, 1027 (1964).
80. L. Ozzello and J. Y. Bembry, *Nature* **203**, 80 (1964).
81. V. I. Oyama, H. G. Steinman, H. Eagle, *J. Bacteriol.* **65**, 609 (1953).
82. M. C. Glick and F. E. Stockdale, *Biochim. Biophys. Acta* **83**, 61 (1964); W. Halle, *Naturwissenschaften* **10**, 234 (1960); I. Harary and B. Farley, *Science* **131**, 1674 (1960); W. Hung and T. Winship, *Proc. Soc. Exptl. Biol. Med.* **116**, 887 (1964); R. E. Priest and J. H. Priest, *Science* **145**, 1053 (1964).
83. M. Webb and J. D. Biggers, *Biochim. Biophys. Acta* **54**, 249 (1961); J. P. Weniger, *Compt. Rend. Soc. Biol.* **152**, 515 (1958).
84. C. Grobstein, in *The Cell*, J. Brachet and A. E. Mirsky, Eds. (Academic Press, New York, 1959), vol. 1; E. Zwilling, in "Symposium on Normal and Abnormal Differentiation and Development," *Natl. Cancer Inst. Monograph 2* (1960).
85. H. Herrmann, *Science* **132**, 529 (1960); P. Weiss, *J. Cellular Comp. Physiol.* **60**, suppl. 1, 45 (1962).
86. C. Grobstein, *Science* **143**, 643 (1964).
87. M. Harris, *Cell Culture and Somatic Variation* (Holt, Rinehart & Winston, New York, 1964).
88. T. C. Hsu, *Intern. Rev. Cytol.* **12**, 69 (1961).
89. V. Defendi, J. Lehman, P. Kraemer, *Virology* **19**, 592 (1963).
90. T. C. Hsu, *J. Natl. Cancer Inst.* **25**, 927 (1960); G. E. Foley and B. P. Drolet, *Cancer Res.* **24**, 1461 (1964); G. E. Foley, A. H. Handler, P. M. Lynch, S. R. Wolman, C. S. Stulberg, H. Eagle, *Cancer Res.*, in press.
91. H. F. Stich, in *Can. Cancer Conf.* **5**, 99 (1963).
92. R. H. Bottomley, H. C. Pitot, H. P. Morris, *Cancer Res.* **23**, 392 (1963); R. H. Bottomley, H. C. Pitot, V. R. Potter, H. P. Morris, *ibid.*, p. 400; T. Ono, V. R. Potter, H. C. Pitot, H. P. Morris, *ibid.*, p. 385; H. C. Pitot, V. R. Potter, H. P. Morris, *ibid.* **21**, 1001 (1961); H. C. Pitot and H. P. Morris, *ibid.*, p. 1009; V. R. Potter, *ibid.* **24**, 1085 (1964); V. R. Potter, in *Cellular Control Mechanisms and Cancer* (Elsevier, Amsterdam, 1964), p. 190.
93. E. Reich, R. M. Franklin, A. J. Shatkin, E. L. Tatum, *Proc. Natl. Acad. Sci. U.S.* **48**, 1238 (1962).
94. D. Nathans, *ibid.* **51**, 585 (1964).
95. H. Eagle and G. E. Foley, *Cancer Res.* **18**, 1017 (1958); "Cancer Chemotherapy Reports," *Cancer Chemotherapy National Service Center Publs.*
96. W. Szybalski and V. N. Iyer, *Federation Proc.* **23**, 946 (1964); A. S. Weisberger and S. Wolfe, *ibid.*, p. 976; A. Falachi and A. Kornberg, *ibid.*, p. 940.
97. E. Klein, *Exptl. Cell Res.* **22**, 226 (1961).
98. R. T. Schimke, *J. Biol. Chem.* **239**, 136 (1964).
99. R. P. Cox and C. M. MacLeod, *Nature* **190**, 85 (1961); ———, *J. Gen. Physiol.* **45**, 439 (1962); G. Melnykovych, *Biochem. Biophys. Res. Commun.* **8**, 81 (1962); H. M. Nitowsky and F. Herz, *ibid.* **11**, 261 (1963).
100. J. J. Maio and L. DeCarli, *Biochem. Biophys. Res. Commun.* **11**, 335 (1963).
101. R. P. Cox and C. M. MacLeod, *Proc. Natl. Acad. Sci. U.S.* **49**, 504 (1963).
102. R. P. Cox and G. Pontecorvo, *ibid.* **47**, 839 (1961).
103. J. B. Alpers, in "Metabolic Control Mechanisms in Animal Cells," *Natl. Cancer Inst. Monograph 13* (1964); ———, R. Wu, E. Racker, *J. Biol. Chem.* **238**, 2274 (1963).
104. H. L. Ennis and M. Lubin, *Biochim. Biophys. Acta* **68**, 78 (1963).
105. J. F. Henderson, *J. Biol. Chem.* **237**, 2631 (1962).
106. I. Lieberman, *ibid.* **2**, 883 (1957).
107. J. Paul, P. F. Fottrell, I. Freshney, W. R. Jondorf, M. G. Struthers, in "Metabolic Control Mechanisms in Animal Cells," *Natl. Cancer Inst. Monograph 13* (1964).
108. E. Bresnick and G. H. Hitchings, *Cancer Res.* **21**, 105 (1961).
109. L. I. Pizer, *J. Biol. Chem.*, in press.
110. H. V. Rickenberg, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 366 (1961).
111. E. McFall and B. Magasanik, *J. Biol. Chem.* **235**, 2103 (1960); D. P. Nierlich and E. McFall, *Biochim. Biophys. Acta* **76**, 469 (1963).
112. J. L. German, III, V. J. Evans, J. A. Cortner, B. B. Westfall, *J. Natl. Cancer Inst.* **32**, 681 (1964).
112a. S. Granick, *J. Biol. Chem.* **238**, PC 2247 (1963).
112b. H. Moser, *Experientia* **16**, 385 (1960).
113. R. Schindler, *Biochem. Pharmacol.* **1**, 323 (1958); R. Schindler, M. Day, G. A. Fischer, *Cancer Res.* **19**, 47 (1959).
114. H. Green and D. Hammerman, *Nature* **201**, 710 (1964).
115. H. Grossfeld, *ibid.* **196**, 782 (1962).
116. J. A. Burdman and M. N. Goldstein, *J. Natl. Cancer Inst.* **33**, 123 (1964).
117. K. W. Thompson, M. M. Vincent, F. C. Jensey, R. T. Price, E. Schapiro, *Proc. Soc. Exptl. Biol. Med.* **102**, 403 (1959).
118. R. T. Jordan, S. Katsh, N. de Stackelburg, *Nature* **192**, 1053 (1961).
119. F. Reusser, C. G. Smith, C. L. Smith, *Proc. Soc. Exptl. Biol. Med.* **109**, 375 (1962).
120. The as yet unpublished work from this laboratory discussed in this article was carried out under National Institutes of Health research grant AI-04153. In its early phases the work program was supported also by National Science Foundation grant G-17192.

The Scientist in the Federal Service

The federal government has become the nation's leading employer of scientists and engineers.

John W. Macy, Jr.

In August of 1964 the Civil Service Commission issued new salary schedules for scientists and engineers in the federal service, setting pay rates for many professional engineering, scientific, and medical positions even above the newly enacted rates of the general salary schedule. The Commission took this action under the authority of the Federal Salary Reform Act of 1962, on the basis of a decision that the higher salaries were necessary to meet nongovernmental pay standards in occupations in which there is a shortage of manpower.

This singling out of science for special attention is not new in the federal government. Salary reform of the past 2 years caps a decade of legislative and administrative actions that have been taken to keep the government's personnel system responsive to the requirements of science and technology. Within the last 10 years employment conditions for the scientist in the federal service have been radically improved.

Ten years ago there were 56,700 federal employees in the physical and biological sciences and 60,500 in engineering, altogether making up 9 percent of the federal white-collar work force. Now there are more than 71,000 in science and 188,000 in engineering, constituting 11½ percent of the white-collar work force. Moreover, from 1951 to 1962 federal employment of white-collar workers increased by 28 percent, while employment of blue-collar workers decreased by 19 percent.

The impact of science and technology has changed the whole makeup of the federal civil service, and is still changing it, day after day. Today there are more professional physical scientists than general clerks, and more engineers than typists.

Scientific research and development are carried out in 25 federal departments and agencies, principally in the

Mr. Macy is chairman of the United States Civil Service Commission, Washington, D.C. He is a member of the President's Science Advisory Committee, and was a member of the former National Academy of Science Committee for Utilization of Scientific and Engineering Manpower.