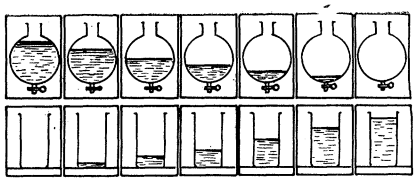

Fig. 4. The correct answer to part II of the test.

ter factory methods, people who have arranged life in their heads in poetic patterns and who have not been told that this is the "wrong" answer. Recently I saw a group of educated men and women who had been presented with some simple problems in building manifolds by means of brightly colored units; the men classified the exercise as "art" and, although they were much better in mathematics and science in college than the women, failed, while the women, who also classified the exercise as "art," at which they thought themselves good, succeeded easily. By failing to cultivate both sides of the child's ability, by opposing them and negating one or the other, we are losing not only artists but also scientists, and we are splitting our society, as well as our individual children, into incompatible parts, destructively at war with each other. A different type of education, which recognizes the early stage in which children can apprehend form through color and kinesthetic feel and the recognition of sets, is a precondition for preserving the creativity with which each generation of newborn children enters the world.

A Whole View of the Past

The second necessary condition, a knowledge of what men have done before, again involves the presentation of wholes—not the current split between the history of science and technology, on the one hand, and art museums and literature courses, on the other. In real life the imagination of the painter and the poet are essential to the conditions within which the scientist works, for the fearful presage of the poet reaches ahead of invention. A few years ago an attempt was made to design an exhibition which would show the effect upon painting of modern scientific invention in building design; but in looking at the materials it was discovered that in every case the painter's vision had preceded the necessary technological invention, as the myth of Icarus preceded the Wright brothers. So we need arrangements which will bring together, for the experience of the student and the adult, whole historic periods—their buildings and their ideas, their books and their economics, their painting and their technology, their mathematics and their poetry—so that out of the perceived relationships and comparisons among them new ideas may be born and the present ignorance among scientists of man's past and present greatness, surpassed only by the ignorance among most humanists and many artists of man's future, made possible by science, may be overcome.

"Chairs of the Future"

Finally, it seems to me, in this age when the very survival of the human race and possibly of all living creatures depends upon our having a vision of the future for others which will command our deepest commitment, we need in our universities, which must change and grow with the world, not only chairs of history and comparative linguistics, of literature and art—which deal with the past and sometimes with the present—but we need also Chairs of the Future, chairs for those who will devote themselves, with all the necessary scholarship and attention, to developing science to the full extent of its possibilities for the future, and who will devote themselves as faithfully to the fine detail of what man might very well—in the light of all our knowledge—be as any classicist or medievalist devotes himself to the texts of Pindar and Horace or to the thought of St. Thomas Aquinas.

Bibliography

M. Carstairs, *The Twice Born* (Hogarth, London), in press.

E. Cobb, "The ecology of imagination in childhood, unpublished.

E. H. Erikson, *Childhood and Society* (Norton, New York, 1950).

L. K. Frank, "Imagination in education," in *Imagination in Education*, Proceedings of the Bank Street College Conference (Bank Street College of Education, New York, 1956), pp. 64–72.

A. Gesell and F. Ilg, *Infant and Child in the Culture of Today* (Harper, New York, 1946).

W. Grey-Walter, *The Curve of the Snowflake* (Norton, New York, 1956).

G. R. Harrison, *What Man May Be, The Human Side of Science* (Morrow, New York, 1956).

G. Hendrix, "Prerequisites to meaning," in *Math. Teacher* 43, 334 (1950).

M. Lowenfeld, "The world pictures of children, a method of recording and studying them," *Brit. J. Med. Psychol.* 18, pt. 1, 65 (1939); "Poleidoblocs" (forthcoming series of tests on children's mathematical imagination; available at the Institute for Child Psychology, 6 Pembridge Villas, London W. 11).

M. Mead, "Arts in Bali," in *Yale Rev.* 30, 335 (1940); "On the implications for anthropology of the Gesell-Ilg approach to maturation," in *Am. Anthropologist* 49, 69 (1947); "Some relationships between social anthropology and psychiatry," in *Dynamic Psychiatry*, F. Alexander and H. Ross, Eds. (Univ. of Chicago Press, Chicago, 1952), pp. 401–448; "Cultural discontinuities and personality transformation," in *J. Social Issues* (Kurt Lewin Memorial Award Issue, Suppl. Ser., No. 8).

C. Morris, *Varieties of Human Values* (Univ. of Chicago Press, Chicago, 1956).

J. Piaget, *Le développement de la notion de temps chez l'enfant* (Presses Universitaires de France, Paris, 1946), chap. 1, pp. 5–36.

E. Sewell, *The Field of Nonsense* (Chatto and Windus, London, 1952).

J. Tanner and B. Inhelder, Eds., *Psycho-biological Development of the Child* (International Universities Press, New York, 1956; 1957), vols. 1, 2.

Molecular Growth Requirements of Single Mammalian Cells

Gordon Sato, Harold W. Fisher, Theodore T. Puck

A technique developed in our laboratory for plating single mammalian cells in petri dishes containing nutrient medium permits each cell to grow in isolation to form a macroscopic colony (*1–3*). The method is comparable, both in simplicity and accuracy, to the standard agar plating procedure of quantitative bacteriology. It has been used for demonstration of the existence of mutant human cell strains and for the isolation of clonal cell lines with stable differentiating characteristics (*4*). It has provided accurate and reproducible analysis of growth curves of single mammalian cells under a variety of conditions (*1–3*) and has made possible quantitative studies of the action on them of agents such as ionizing radiation (*5*), antibodies, drugs, and hormones (*6*). This article (*7*) describes the application of this method to study of the molecular growth requirements of mammalian cells.

Procedure and Results

These experiments were carried out with the S3 clonal strain of the HeLa cell (*1*). Elegant studies on massive populations of the parental HeLa strain

The authors are on the staff of the department of biophysics, Florence R. Sabin Laboratories, University of Colorado Medical Center, Denver.

have been published by Eagle and his colleagues (*8*), who reported that the organic nutritional needs of these cells, as of other cells of human origin, were satisfied in mass culture by a medium containing 13 amino acids, eight vitamins, and glucose, supplemented with the dialyzed macromolecular portion of human serum in an amount equivalent to 10 percent of whole serum. Our own studies with single cells have revealed some interesting differences from the behavior of large populations and have shown that the molecular requirements for growth displayed by human cells may vary tremendously, depending on a variety of conditions, some of which it is the purpose of this article to delineate.

In an earlier publication we demonstrated that interaction between cells in a large population could relieve the need for a specific molecular species in the growth of a given cell type. Thus, the HeLa S3 strain requires inositol when it is plated as single cells on a petri dish, but it grows with 100-percent efficiency and maximal growth rate in the absence of inositol if it is plated on top of S3 cells whose own reproductive capacity has been previously inactivated by x-irradiation (*9*). Similarly, we have shown that media sufficient to promote growth for many months of human fibroblastic cells in massive culture are incapable of promoting colony formation when isolated cells are plated unless a feeder layer, or enriching nutrient substances, is supplied (*3*).

Earlier experiments from our laboratory also have demonstrated the genetic heterogeneity of standard tissue-culture stocks and have revealed that clonal cell strains which show marked differences in their nutritional requirements, when they are grown as single cells (*4*) can be isolated from these stocks. These differences have maintained themselves in the respective stocks throughout continuous cultivation in the same medium for several years and for hundreds of generations. While the nutritional requirements of single HeLa cells of the S1 clonal stock are distinctly different from those of S3, and from the parental HeLa culture, which seems to contain a preponderance of S3-like forms, evidence was presented that these differences may be masked in massive cultures. When stocks which consist of mixtures of genetic constitutions are employed, the composition of the different genotypes in the population may be expected to change in the course of time in any particular laboratory, and along different paths in laboratories which originally began with the same population, because of the different selection processes that may operate in different places.

The basal synthetic medium used in all of our experiments (*2, 3*) is shown in Table 1 and includes the components designated by Eagle as essential. Routine cultivation of our cells is carried out in this basal medium, supplemented with 16 percent human serum and 8 percent horse serum. Experiments revealed that the basal synthetic medium of Table 1 is adequate for growth into colonies of single S3 cells if it is supplemented with 10 to 20 percent of whole human serum, or with the dialyzed, macromolecular portion of serum. In the latter case, however, the effectiveness of the resulting medium was found to be sharply dependent on the dialysis procedure employed. Dialysis of fresh human serum against Hank's saline for moderate periods of time (3 to 4 hours) results in a macromolecular preparation whose potency in supporting cell growth, when it is added to the basal medium, approximates that of whole serum. However, if fresh human serum is subjected to prolonged dialysis, accompanied by several changes of the external medium, or if an aged serum is employed in a mild dialysis procedure, the resulting macromolecular material loses its ability to confer growth potency to the basal synthetic medium described (Table 2).

Table 1. Composition of the basal synthetic medium which has been used in the experiments. This solution is diluted to 40 percent in the final medium, which also contains serum components and any other growth factors which may be desired. Final adjustment of the volume is made with Hank's saline.

Component	Amount (g/lit)
L-Arginine	0.0375
L-Aspartic acid	0.0300
L-Cystine	0.0075
L-Glutamic acid	0.0750
Glycine	0.100
L-Histidine	0.0375
DL-Isoleucine	0.0250
L-Leucine	0.0250
L-Lysine	0.0800
L-Methionine	0.0250
β-Phenyl-L-alanine	0.0250
L-Proline	0.0250
L-Threonine	0.0375
L-Tryptophan	0.0200
L-Tyrosine	0.0400
DL-Valine	0.0500
Biotin	0.00010
Calcium pantothenate	0.0030
Choline	0.0030
Folic acid	0.00010
Niacinamide	0.0030
Pyridoxine	0.00050
Riboflavin	0.00050
Thiamin	0.0050
Glutamine	0.200
Hypoxanthine	0.0250
Glucose	1.20
Phenol red	0.0125
NaCl	7.00
KCl	0.20
$CaCl_2 \cdot 2H_2O$	0.14
$MgCl_2 \cdot 6H_2O$	0.10
Na_2HPO_4	0.23
KH_2PO_4	0.10
$NaHCO_3$	2.24
Penicillin	0.250
Streptomycin	0.250

Table 2. Demonstration that prolonged dialysis of human serum results in loss of its ability to render the synthetic nutrient solution (Table 1) capable of supporting growth of S3 cells. Human serum was placed inside a cellophane bag and dialyzed with rapid agitation against nine volumes of Hank's saline for 4 hours, a period sufficient to produce equilibration of free, small molecules throughout the aqueous phase. A sample of the macromolecular portion was withdrawn, a fresh charge of saline was placed outside the bag, and dialysis was repeated. Five successive dialysis operations of this kind were performed, and the corresponding samples of the macromolecular portion of serum were then tested for their ability to promote the growth of S3 cells when added to the synthetic solution described in Table 1.

No. of standard dialyses to which the serum protein was subjected	Plating efficiency (Percentage of cells yielding colonies of 100 or more cells after 14 days' incubation in 5% Co_2)
0 (whole serum)	83
1	74
2	0
3	0

Table 3. Composition of the supplement to the nutrients listed in Table 1, which can produce growth of single S3 cells, even when the macromolecular serum constituents have been subjected to excessive dialysis. All of these materials have been incorporated in mixtures proposed for cultivation of various cell types, and in particular, in the medium, NCTC(108) (*10, 15*).

Component	Amount (mg/100 ml)
Cholesterol*	0.08
Ascorbic acid	2.0
Coenzyme A	0.10
Co-carboxylase	0.04
Diphosphopyridine nucleotide	0.04
Flavinadenine dinucleotide	0.04
Glutathione	0.40
Cysteine	10.0

* Cholesterol was employed as an aqueous suspension dispersed by means of Tween 80 and alcohol, in accordance with the procedure of Earle and his colleagues (*10*). The final growth medium contained 0.04 mg of Tween 80 per liter. Tween alone was not effective.

Experiments were initiated to determine whether growth competence could be restored to such preparations by the addition of defined molecular species. The test was carried out first on various substances known to be important in mammalian metabolic reactions, and some of which have been routinely incorporated into solutions designed to support cell growth in tissue culture (*10, 11*). Such experiments revealed that growth competence which has been lost by prolonged dialysis can be restored by the addition of specific nutrilites to the medium.

The number of different molecular species required to restore growth to such a medium varies with the serum specimen employed and its treatment. However, a solution containing the ingredients listed in Table 3 was found uniformly to produce growth of single cells, even after repeated dialyses.

While this supplementary group of molecules appears sufficient to restore growth competence to any batch of serum macromolecules of our experience, not all of these additional substances are equally necessary, some appearing to lend only small support, and others perhaps functioning only to offset other imbalances present in the medium (*12*).

However, the case of cholesterol is of especial interest. The need for this substance is usually one of the first to be revealed when the serum macromolecules are exhaustively dialyzed, and often a considerable degree of growth sufficiency can be restored by addition of the cholesterol-Tween suspension alone. Figure 1 illustrates the appearance of a plate seeded with 100 single cells which were grown in the presence of cholesterol. In the absence of cholesterol, a virtually blank plate results. This effect can be quantitatively titrated by adding to a series of identical plates varying amounts of the standard cholesterol suspension. A typical curve so obtained is exhibited in Fig. 2; it resembles the relationships obtained in bio-assay procedures with bacterial cells.

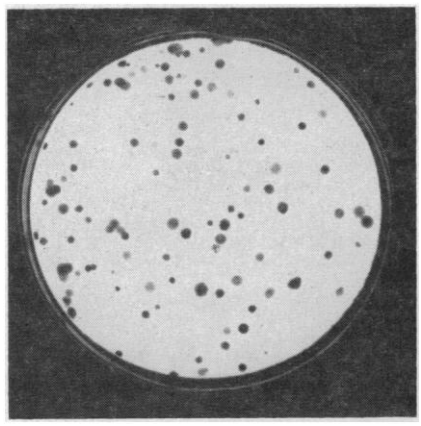

Fig. 1. Demonstration that the addition of a Tween 80-alcohol suspension of cholesterol restores to a highly dialyzed serum the ability to support growth of S3 cells. One hundred S3 cells were added to each of a series of plates containing the medium listed in Table 1, to which was also added ascorbic acid, coenzyme A, and co-carboxylase as in Table 3, and a highly dialyzed preparation of the macromolecular constituents of serum, in an amount equivalent to 15 percent of whole serum. The plate shown received a Tween-alcohol cholesterol suspension in an amount to make the final cholesterol concentration 2 micrograms per milliliter. The plates were fixed and stained after 10 days of incubation. On three identical plates with cholesterol, 105, 104, and 103 well-defined colonies appeared. On three similar plates without cholesterol, the counts were 0, 2, and 1, respectively, and these colonies were so small that they were barely visible.

Discussion

The simplest explanation of these results is that the serum proteins tightly bind small molecular substances which are essential for growth of cells such as we have studied. Prolonged dialysis, possibly accompanied by some denaturation, releases the molecules, which then must be supplied from external sources. One of the functions of serum proteins in supporting growth of cells *in vitro* would thus appear to serve as a reservoir of such essential, small molecules. Recent experiments (12*a*) have shown that the macromolecular constituents of serum needed for growth of single S3 cells include albumin, which strongly binds small molecules, and a globulin fraction exhibiting a single electrophoretic peak with a mobility of -4.4×10^5 square centimeters per second, per volt at *p*H 8.5.

These experiments illustrate how single-cell techniques can be used to uncover nutritional needs of mammalian cells, needs which are not readily demonstrable by other methods. They also demonstrate how the apparent molecular growth requirements of a given cell may be greatly influenced by the experimental conditions. The ability of cells growing in close juxtaposition to one another to exert strong influences on one another's growth was emphasized by Earle and his co-workers (*13*) and demonstrated in specific ways by our own experiments on feeder cell systems (*9*). The mechanism by which a cell growing in a community can dispense with certain molecules essential for growth of single cells constitutes a problem of fundamental importance. It is possible, as Earle has suggested, that all or most cells possess minimal biosynthetic capacities for many molecules which are lost too rapidly from isolated cells to permit initiation of cell reproduction. Moreover, the permeability of cells closely packed in a colonial array may conceivably be markedly different from that characteristic of isolated cells. These considerations could also explain the much greater difficulty exhibited in obtaining growth of fibroblastic, as compared with epithelial-like, cells (*3*), for the more highly stretched condition of the former exposes more surface, and their greater tendency to form loose, open meshworks, instead of tight, compact masses, would encourage greater loss of diffusible, essential metabolites.

Other factors also can exert strong influences on the apparent growth requirements of mammalian cells. One of these is the incubation period which is permitted for colonial development of single cells. Often a medium will seem adequate for growth, permitting colony formation to the number of 50 or even 100 cells. On continued incubation, however, the number of cells per colony will become stationary and then will fall, as the cells begin to disintegrate. Results of this type are undoubtedly influenced by the cells' ability to store large quantities of needed metabolites. We have also found that the medium of previous cultivation can profoundly influence experiments testing the growth of single cells. Finally, we have observed that certain molecules appear to be necessary for growth of single cells only when other substances are also included in the medium, an indication of the need to maintain certain metabolic balances (*14*).

Summary

The molecular nutritional requirements exhibited by mammalian cells can vary with the following conditions: whether the cells are plated singly or in massive inoculum; whether a clonal strain or a mixture of different genotypes is employed; whether the macromolecular fraction of serum used as a supplement was previously exposed to pro-

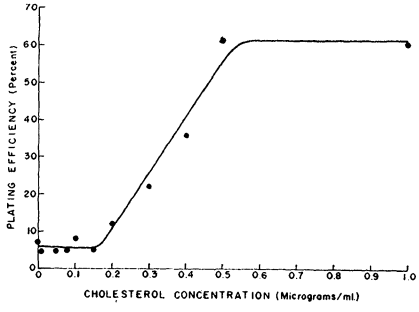

Fig. 2. Titration curve showing the quantitative relationship between the plating efficiency of single HeLa S3 cells and the amount of cholesterol-Tween 80 suspension added.

longed dialysis or other procedures which may release bound micromolecules. In addition, the nature of the medium in which the cells were previously grown, the time permitted for incubation of the cells in the test medium, and the presence of substances or conditions which may specifically introduce the need for certain molecules to preserve balance can strongly influence nutritional requirements. This great versatility may reflect the ability of the mammalian cell to assume different metabolic states characteristic of specific types of differentiation. Under certain conditions, growth of single HeLa cells of the S3 clonal strain requires the presence of cholesterol at a level of 0.5 to 1 microgram per milliliter.

References and Notes

1. T. T. Puck, P. I. Marcus, S. J. Cieciura, *J. Exptl. Med.* **103**, 273 (1956).
2. P. I. Marcus, S. J. Cieciura, T. T. Puck, *ibid.* **104**, 615 (1956).
3. T. T. Puck, S. J. Cieciura, H. W. Fisher, *ibid.* **106**, 145 (1957).
4. T. T. Puck and H. W. Fisher, *ibid.* **104**, 427 (1956).
5. T. T. Puck and P. I. Marcus, *ibid.* **103**, 653 (1956).
6. T. T. Puck *et al.*, in progress.
7. This article is contribution No. 61 from the department of biophysics, University of Colorado Medical School. This research was supported by grants from the Commonwealth Fund and the Rockefeller Foundation.
8. H. Eagle, *J. Biol. Chem.* **214**, 839 (1955).
9. H. W. Fisher and T. T. Puck, *Proc. Natl. Acad. Sci. U.S.* **42**, 900 (1956).
10. V. J. Evans *et al.*, *Cancer Research* **16**, 87 (1956).
11. J. F. Morgan, H. J. Morton, R. C. Parker, *Proc. Soc. Exptl. Biol. Med.* **73**, 1 (1950).
12. Detailed studies of the role and degree of specificity of each of these substances are in progress.

12*a*. H. W. Fisher, T. T. Puck, G. Sato, in preparation.

13. K. K. Sanford, W. R. Earle, G. D. Likely, *J. Natl. Cancer Inst.* **9**, 229 (1948).
14. A paper describing the details of these effects is in preparation.
15. Samples of medium NCTC(108) were kindly supplied to us by Wilton Earle.

E. S. G. Barron, Medical Biochemist

E. S. Guzman Barron, professor of biochemistry in the Department of Medicine at the University of Chicago, died 26 June 1957, at the age of 58. He leaves a host of friends in science, scattered throughout the world. He was educated in Peru, his native country, and completed his medical education at the University of San Marcos at Lima, in 1924. He then spent 2 years of study in Paris and Strassburg. In 1927 he came to the United States to work with the late Leonor Michaelis at the Johns Hopkins Medical School.

It was there that he began his distinguished work on the role of sulfhydryl compounds in biological systems—work that he continued throughout his career. This was also the period during which he made the original observation that nonrespiring cells, such as mammalian erythrocytes, can be converted to actively respiring cells by the addition of a suitable oxidation-reduction mediator, such as methylene blue.

In 1930 he came to the University of Chicago and became assistant professor in the Department of Medicine in 1931, associate professor in 1942, and professor in 1952. He was a member of the research staff of the Lasker Foundation for Medical Research at the University of Chicago.

During these 27 years, Barron made pioneering and consistently significant contributions to the problem of biological oxidations. Among these were studies on the mechanism of lactic acid oxidation by bacteria, the oxidation-reduction potential of the lactate-pyruvate system, the oxidation-reduction potentials of hemochromogens, and the key position of pyruvate in intermediary metabolism. His article in *Physiological Reviews,* in 1939, on cellular oxidation systems is a classic which well merits careful study today.

Except for the war years, when he engaged in research for the Office of Scientific Research and Development, Barron made continual contributions to what we now know about intermediary metabolism of foodstuffs. His biochemistry was ultimately oriented toward the metabolic behavior of tissues and the part that individual enzyme systems play in over-all metabolism.

During and after the war, Barron actively engaged in the study of the biochemical mechanism of radiation damage. In this work he was a proponent of the important part played by sulfhydryl groups and of their modification through radiation.

Although he did little formal teaching, he had a continuous flow of post-doctoral fellows in his laboratory—men from many fields of medical science and from many countries. His influence as a teacher of those who are today's investigators in the medical sciences was very great indeed. And, to all, he imparted the excitement of curiosity, the value of industrious documentation, and the importance of critical evaluation. His younger brother, Alberto Guzman Barron, was one of those who received inspiration aand biochemical training under his guidance; he now holds the chair of biochemistry and nutrition at the University of San Marcos at Lima.

Personally, Achito, as those of us who knew him affectionately called him, was serious and single-minded in all his scientific activities, but apart from these, he was gay and full of the joy of living.

He loved Woods Hole and always spent his summers there, where he was a trustee of the Marine Biological Laboratory. As he often stated, it was through his contacts at the Marine Biological Laboratory that he broadened his outlook and received inspiration for his winter's research activities.

In Peru, although he became an American citizen in 1939, he is recognized as one of the leading scientists of the country. There he has been honored by being made honorary professor of the University of San Marcos at Lima and by being awarded an honorary Doctor of Science degree, by the University of Trujillo, and the Order Del Sol.

I visited his laboratory on two occasions last spring and I found it a beehive of productive activity, with Achito the hardest worker of them all. It is tragic, indeed, that he is lost to science and to the scientific community at the height of his productivity. But still more to be regretted is the loss of his warm personality from the company of those who bore him great admiration and affection.

A. Baird Hastings
Harvard University Medical School