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INSTRUMENTS AND TECHNIQUES

Animal Cell Cultures

Tissue culture is a powerful tool in the study of nutrition, physiology, virology, and genetics.

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The usefulness of animal cell cultures as an experimental tool in such diverse fields as biochemistry, biophysics, genetics, virology, and cancer research has become increasingly evident in recent years. Nevertheless, many workers have been deterred from using this tool by the belief that the technique presents formidable manipulative difficulties and requires a heavy commitment of time for routine maintenance of cultures. Perhaps these were once important considerations. However, with the simplified procedures now available, animal cell cultures represent a technically simple biological system of major importance.

In this article I describe the simple and reliable cell-culture techniques presently being used in this laboratory which have made possible biochemical, genetic, and virus studies. Many of the specialized procedures used for cell propagation or for the initiation of primary cell cultures are not described, since this information is contained in several books and articles which have recently been published (*1*). Some of the findings are discussed, and a few of the present uses of cell cultures are cited.

In general, the manipulations involved in cell culture are similar to those employed in a bacteriological laboratory. The various fluids must be sterilized, as must all the glassware, and aseptic technique must be used. The addition of penicillin and streptomycin to the medium greatly facilitates the maintenance of sterile cultures. Maintaining duplicate stock cultures, which are fed with separate batches of media, is a further protection against loss of stocks due to contamination.

Perhaps the best assurance against the loss of strains is a repository of frozen stock cultures. Cells frozen in a serum containing growth medium supplemented with 5 to 20 percent glycerol and stored at −70°C yield viable cultures, when thawed, even four years later (*2*, *3*). Stocks grown from frozen cultures may further serve as controls for cultures which have been serially propagated for long periods and in which a new cell type may have emerged as a result of genetic selection.

Culture Media

A major development in recent years has been the replacement of growth media containing ascitic fluid, serum, plasma, and tissue extracts by simple, chemically defined media supplemented with low concentrations of dialyzed serum. This has permitted meaningful and reproducible biochemical experiments. The composition of the basal medium developed by Eagle and his associates, currently in use in this laboratory, is shown in Table 1 (*4*). This medium will support the growth of monolayer and suspension cultures of both normal and malignant cells, most cell cultures growing with an 18- to 26-hour generation time. It has also been used in the establishment of primary cultures from human biopsy material (*5*). Each of the components present in it has been demonstrated to be essential for growth, and the omission of any single component will result in cell death. The six amino acids glycine, alanine, serine, glutamic acid, aspartic acid, and proline, which are not components of the medium, can be shown to be formed *de novo*, the first three deriving their carbon atoms from glucose and the latter three, from glutamine (*4*). Similarly, the purine and pyrimidine components of ribonucleic acid and deoxyribonucleic acid can be formed *de novo* (*4*, *6*). With most serially propagated strains, the addition to the medium of the six nonessential amino acids or purines or pyrimidines or their derivatives does not significantly stimulate growth. However, their presence may alter the enzymatic composition and potential of these cells, their presence acting to suppress those enzymes involved in their *de novo* formation (*7*).

Among the essential amino acids only glutamine is interconverted and thus utilized for the biosynthesis of other cellular components. Its carbon skeleton serves as the primary source for the synthesis of the pyrimidines (*8*) and of glutamic acid, aspartic acid, asparagine, and proline (*4*). Further, its amide nitrogen is utilized directly as a source of purine and pyrimidine nitrogen (*8*), and its amino nitrogen serves as the source of the amino nitrogen of the nonessential amino acids (*4*). The failure to detect significant levels of incorporation into the nonessential amino acids when various uniformly C^{14}-labeled essential amino acids are present in the medium suggests that the latter are not degraded to any signifi-

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cant extent. It is not clear whether those enzymes involved in the catabolism of the essential amino acids are present but fail to express themselves, whether these enzymes are diluted out in a rapidly growing culture, or whether, in the establishment of primary cultures, cells containing these enzymes are at a selective disadvantage and are rapidly eliminated from the culture.

The classification of amino acids as essential relates only to cell growth and survival and is not informative at the enzymatic level. At least one of the "essential" amino acids, cysteine, can be synthesized *de novo* from methionine and glucose but is still required for cell survival, suggesting that its rate of biosynthesis is insufficient to permit either cellular proliferation or maintenance of cell viability (*9*). For other amino acids—namely, threonine, valine, leucine, isoleucine, glutamine, arginine, histidine, lysine, methionine, phenylalanine, and tyrosine—the present indication is that the requirement reflects the inability of the cell to synthesize these components *de novo*. However, citrulline (but not ornithine) has been shown to substitute for arginine (*4, 10*), and the keto analogs will in most cases substitute for the corresponding essential amino acids, indicating the cell's ability to carry out a limited number of steps which are required in the synthesis of the essential amino acid (*4*). [The ability of keto acids to substitute for the corresponding amino acids, however, may only reflect the lack of specificity of the transaminases which are present in the cell (*11*) but whose primary role is in the synthesis of the nonessential amino acids.]

Whether the failure to synthesize these essential amino acids is the result of single or multiple metabolic blocks is at present not known. While identical amino acids are required for the growth of a variety of normal and malignant cells (*12*), it is nevertheless possible that the specific metabolic blocks in the synthesis of a particular amino acid differ from cell to cell; and under these circumstances one might find syntrophism between different cell lines in a medium lacking a single essential amino acid. Even more remarkable than the qualitative similarity in amino acid requirement among various cell lines would be the finding that cultures deriving from normal and malignant tissues of both human and animal species possess identical patterns of biochemical lesions in the synthesis of each of the essential amino acids. It is possible that cultures with discrete biochemical lesions are presently available, and that the presence of such lesions will be revealed by detailed biochemical analysis.

Table 1. Minimum essential medium for cultivation of mammalian cells in either monolayer or suspension cultures. Optional supplementation is of two kinds: (i) nonessential amino acids (glycine, alanine, serine, proline, glutamic acid, aspartic acid, asparagine), each at 0.1 m*M*; (ii) sodium pyruvate (1 m*M*).

Compound	Concentration (m*M*)	Amount (mg/lit.)
L-Amino acids		
Arginine	0.6	105
Cystine	0.1	24
Glutamine	2.0	292
Histidine	0.2	31
Isoleucine	0.4	52
Leucine	0.4	52
Lysine	0.4	58
Methionine	0.1	15
Phenylalanine	0.2	32
Threonine	0.4	48
Tryptophan	0.05	10
Tyrosine	0.2	36
Valine	0.4	46
Carbohydrate		
Glucose	5.5	1000
Salts		
NaCl	116	6800
KCl	5.4	400
$CaCl_2$	1.8 (0)*	200 (0)*
$MgCl_2 \cdot 6H_2O$	1.0	200
$NaH_2PO_4 \cdot H_2O$	1.1 (11)*	150 (1500)*
$NaHCO_3$	23.8	2000
Vitamins		
Choline		1
Folic acid		1
Inositol		2
Nicotinamide		1
Pantothenate		1
Pyridoxal		1
Riboflavin		0.1
Thiamine		1
Others		
Penicillin		50
Streptomycin		50
Phenol red		5
Serum protein		
Supplied as 5- to 10-percent whole or dialyzed serum		

* For suspension cultures.

Serum Requirement of Animal Cell Cultures

Human, equine, and bovine sera have been most commonly used as a medium supplement. Prior to use, the serum may be dialyzed at 5°C for 24 hours. The sodium chloride concentration is then readjusted to 0.85 percent, and the serum is sterilized by filtration through a Selas No. 03 filter and added to the medium at a final concentration of 5 to 10 percent. Since human serum from a particular donor may prove somewhat toxic, sera from ten individual donors are routinely pooled. Such pooled serum has not been found to be toxic. A similar procedure can be followed with sera from other species; alternatively, the serum from a single donor can be pretested to determine its suitability—for example, to support growth, to support virus replication, and so on. The choice of serum species is dictated in part by availability and in part by the type of study being carried out. The possible presence of viral antibodies in human serum, for example, precludes its use for certain virus studies. No significant differences in gross chemical composition or in metabolic behavior have been observed when HeLa cells grown in dialyzed human serum and in dialyzed horse serum were compared. However, striking morphological changes have been observed to be serum-dependent, and reversible (*13*).

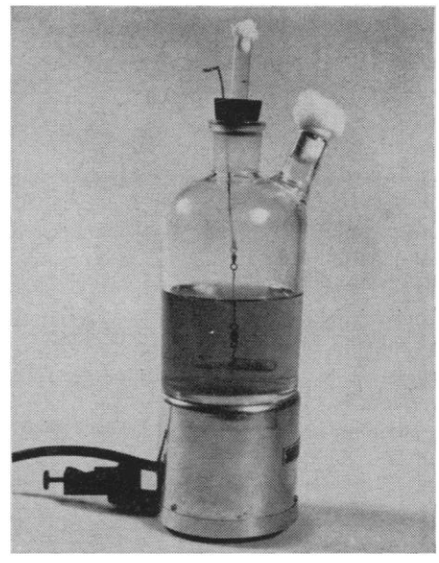

Fig. 1. Assembly for growth of animal cells in suspension.

Dialyzed serum can be stored at 4°C for several months and still remain active in supporting growth. However, proteolytic enzymes which are present in serum will cause hydrolysis on storage even at this temperature, resulting in a significant level of contamination of the serum by peptides and free amino acids (*14*). Breakdown is not detected in serum which is dialyzed, sterilized by filtration, and then stored in the frozen state prior to use.

A great deal of effort has been directed towards understanding the role of serum protein in animal cell nutrition. It has been reported that only two purified fractions are essential for the growth of the HeLa S3 cell, serum

albumin and a glycoprotein (*15*). While a chemically defined medium supplemented with these two protein fractions will support clonal growth of HeLa S3 cells, it does not suffice for the clonal growth of human euploid cell cultures (*16*). Work in other laboratories indicates that neither protein is an absolute requirement for cell attachment or for growth of many cell lines. Variants have been obtained from human and mouse cultures and have been serially propagated in a chemically defined medium by procedures which involve frequent detachment from glass and subsequent reattachment (*17*). In other cultures, where no selection for a particular cell type has occurred, other proteins or factors derived from protein can be substituted for serum. Thus, products released on the digestion of serum by the crude proteolytic enzyme preparation Viokase diffuse through a semipermeable membrane at a rate which permits sustained growth of suspension cultures of all cell lines tested (*18*). Similarly, monolayer cultures have been successfully grown in a medium supplemented with lactalbumin hydrolyzate and salmine (*19*).

The present evidence for mass populations would indicate no absolute requirement for any specific protein. Since serum-free media which support growth have contained varying types of degraded protein—that is, bacto-peptone (Difco), lactalbumin hydrolyzate, or the dialyzable products of Viokase-treated serum—it seems unlikely that they are supplying to the cells an identical protein degradation factor of any significant size. It seems more likely that the contribution of these various peptide products, or of serum, is that of providing a source of material of low molecular weight which is bound to them, and which they gradually release to the cell.

However, recent reports by Rappaport *et al.* (*20*) suggest quite a different role for protein or factors derived from protein. The HeLa, L, and primary monkey-kidney cells were successfully grown in monolayer cultures in a chemically defined medium without a prolonged lag period required for cell adaptation or selection. Successful growth was dependent on proper pretreatment of the glass surface with alkali. The data indicate that such treatment modifies the net charge on the glass surface, and further, by establishing a reservoir of Na^+ ions, permits continued cell attachment even with the diffusion of protons from the cells which occurs as a result of cell growth and metabolism. These results suggest that protein does not have a nutritional role but, rather, is important in producing a physically compatible cell surface, which is required in both monolayer and suspension cultures and which, in the former, permits attachment of cells to the glass.

Establishment of Cell Cultures

Well over 100 cultures have been successfully initiated from tissues, both normal and malignant, of a number of animal species and maintained in a rapidly growing state for prolonged periods. The procedure for initiating cultures involves mincing of the tissue, treatment with a proteolytic enzyme (usually trypsin) in order to disperse large fragments and liberate single cells, and inoculation of the tissue digests into a suitable growth medium. Procedures have been reported which permit the establishment of cultures from extremely small amounts of tissue with consistent success (*21*). Moreover, subcultures have remained euploid over a period of one year, during which time they were maintained in an actively growing state. Unfortunately, with few exceptions, the specialized functions characteristic of the organ of origin are not present in the serially propagated culture. For reasons which are not yet clear, cultures initiated with cells derived from liver do not convert phenylalanine to tyrosine, nor does the mouse fibroblast culture produce collagen.

I indicated previously that many cultures had been compared and that identical nutritional requirements were demonstrated, regardless of the origin of the culture. However, when cultures were compared for their ability to produce tumors in the cheek pouch of the hamster, cultures derived from malignant tissues could be distinguished from cultures derived from normal tissues; the latter produced tumors only when 100 times as many cells were injected as were required for tumor production with cells from cultures derived from malignant tissue (*22*). In this study there was no indication that cultures derived from normal tissues regularly become malignant after prolonged maintenance under conditions of rapid growth. These studies further argue against the idea that all cultured cells dedifferentiate into a common, primitive cell type.

The similarity in nutritional requirements of serially propagated cultures may depend on the fact that the techniques which are used in establishing

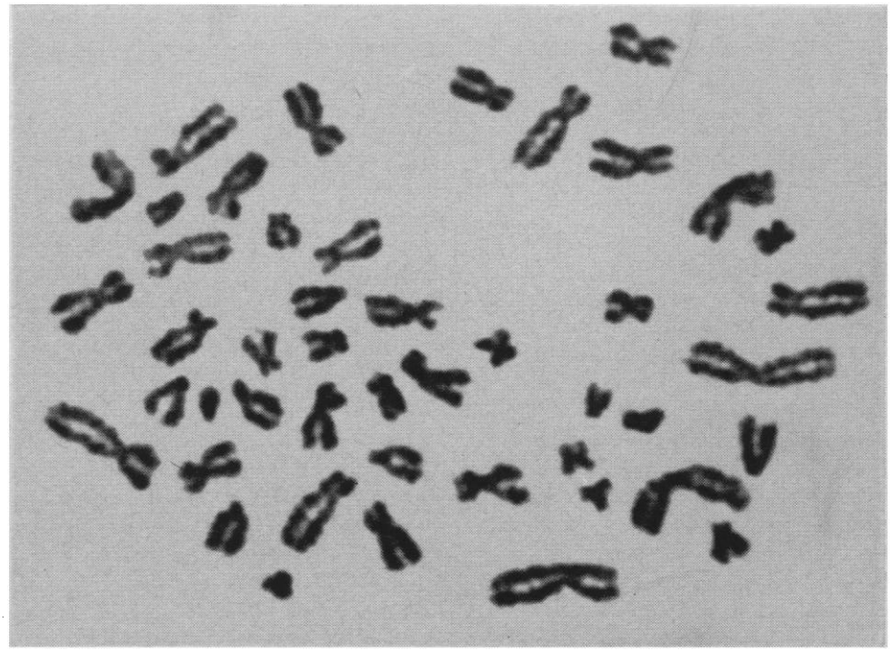

Fig. 2. The chromosomes of human lung fibroblasts grown in vitro. The cultures were primary explants, carried out by Rune Grubb (Institute of Bacteriology, Lund, Sweden), of tissue taken from human embryos. The chromosomes were studied a few days after the in vitro explanation of the biopsy material, from an acetic orcein squash preparation pretreated with colchicine and hypotonic saline. The photomicrograph [from Tjio and Levan (*35*)] shows an early metaphase. These cultures were the first in which the number of chromosomes in man was found to be 46 and not 48.

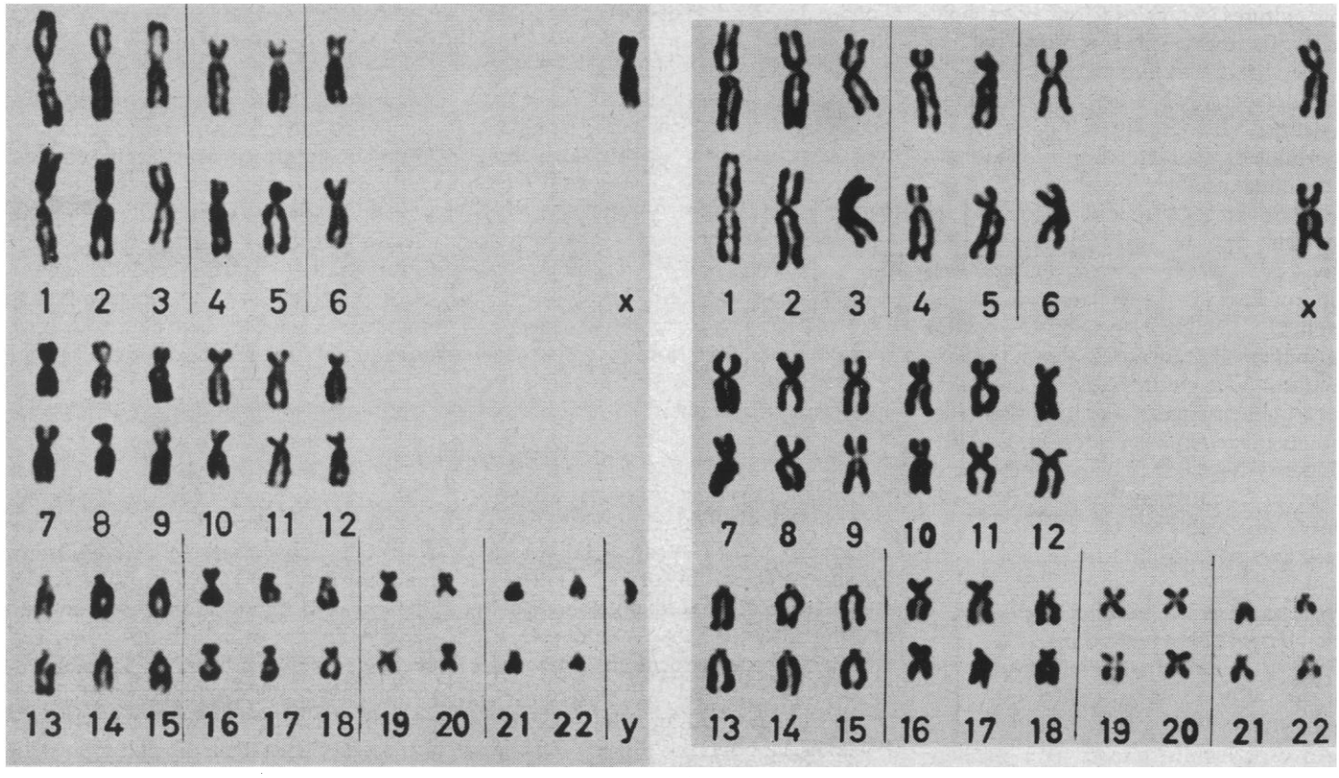

Fig. 3. The human male (left) and female (right) karyotypes. Photomicrographs of the individual chromosomes were cut from enlarged photomicrographic prints (original enlargement, × 4000), paired, and arranged according to a standard system of nomenclature [*Am. J. Human Genet.* **12**, 384 (1960)] (about × 2840). [Courtesy of J. H. Tjio]

them all select for the most rapidly proliferating cell types, cells dividing at a slower rate soon being lost from the population. Methods which would eliminate the rapidly proliferating cells might conceivably allow for the emergence of new types of cell cultures. A possible approach is provided by the observation that 5-bromodeoxyuridine is incorporated into the deoxyribonucleic acid of animal cells, this incorporation finally proving lethal (*23*). The presence of 5-bromodeoxyuridine in the medium for varying periods when cultures are established would result in its preferential uptake by the rapidly growing cell. These cells would then fail to grow when the inhibitor was subsequently removed or when its action was reversed by the addition of thymidine, and thus selection for the more slowly dividing cells could occur.

Techniques for Cell Maintenance

For routine maintenance, cells are grown in stoppered flat-bottom flasks. The culture is divided when the cells form a confluent sheet. Used medium is decanted, and the cells are removed from glass by mechanical scraping with a rubber-tipped policeman into fresh medium, or by treatment with a Versene or trypsin solution (*1*). The first procedure is the simpler for routine maintenance. Clumps are dispersed by pipetting the suspension back and forth, and a suitable aliquot is added to a bottle containing fresh medium. Routinely, 1/10 to 1/20 of the cells is used as an inoculum. These cultures are fed on the third and the fifth day and are again divided on the seventh day. With more frequent feeding a somewhat more rapid rate of cell proliferation can be obtained.

When a series of replicate flasks is required for an experiment, it is desirable to remove cells from glass with either trypsin or Versene. This gives well-dispersed suspensions, which are then centrifuged and resuspended in fresh medium. They are continuously agitated with a magnetic stirrer, and equal aliquots are dispensed with a Cornwall pipet into flasks containing the desired medium. Cells from suspension cultures may also be used to prepare monolayer cultures, and in this case preliminary dispersion of the cells is not required.

Suspension cultures (*24*) can readily be initiated from monolayer cultures with many cell lines, though some cell lines have been found to be unsuitable, due to excessive clumping. Cells from a monolayer culture are dispersed into a medium modified to permit growth of suspension cultures (for its composition, see Table 1) and transferred to a spinner flask (Fig. 1). The rate of stirring is adjusted to maintain a slight vortex at the surface of the liquid. The cultures are initiated at a population of 100,000 cells per milliliter, the volume of fluid being sufficient to keep the bottle almost half full. Lesser volumes permit too rapid a loss of carbon dioxide, while too large a volume results in excess acidity due to trapping of carbon dioxide. The cultures are diluted every second day, and cultures maintained between 1×10^5 and 5×10^5 cells per milliliter can be kept indefinitely in the log phase of growth. External control of the *p*H has not been found necessary for cells grown in this manner.

The behavior and characteristics of monolayer and of suspension cultures are not equivalent, and the particular study may dictate which is to be preferred. Steady-state growth conditions cannot be obtained in monolayer cultures but may be obtained in suspen-

sion cultures, which will remain indefinitely in the logarithmic phase of growth if the cultures are suitably diluted (see above). In monolayer cultures the size of the inoculum will determine the length of time cells can be maintained in the log phase, while the glass area of the bottle in which the cells are grown will determine the maximum cell population that can be achieved.

These monolayer cultures display three distinct phases of growth—a lag, a logarithmic, and a stationary phase. Further, monolayer cultures show systematic fluctuations in their cellular content of deoxyribonucleic acid, ribonucleic acid, and protein, levels of the latter two decreasing sharply with increasing age of the culture (*25, 26*). As would be expected, the relative rates of synthesis of ribonucleic acid and deoxyribonucleic acid, as measured by uptake of C^{14}-labeled nucleic acid precursors, is also dependent on the age of the cultures (*25*). Good reproducibility from experiment to experiment is thus dependent on the age and metabolic state of the culture and may be difficult to achieve. Similar wide variations in response to virus infection have been shown to be dependent on the metabolic state of the culture (*27*). However, the ease with which media can be decanted without disturbing monolayers and subsequently replaced by media containing any desired test substance is a distinct advantage. The techniques available for readily preparing large numbers of replicate monolayer cultures and measuring the changes in cell mass make monolayer cultures ideal where the effects on cell growth of a large number of agents at several concentrations are being evaluated (*28*).

Suspension cultures do provide a source of cells in a highly reproducible metabolic state. Through proper dilution schedules, these cells can be maintained in the logarithmic phase of growth and show a constant content of ribonucleic acid, deoxyribonucleic acid, and protein per cell (*29*). Since aliquots can be removed from a single culture, this obviates the need for planting replicate cultures. Further, cells are collected readily by centrifugation, rather than by the more tedious and traumatic procedures involved in harvesting cells from glass. Finally, cells can be harvested and resuspended at any desired population, so that effects of varying cell population may be evaluated.

Cloning of Mammalian Cells

The technique for the production of clones from single cells, as originally described, required x-irradiated nondividing cells which served as a feeder layer and which permitted the formation of clones from single viable cells (*30*). Subsequent studies, however, demonstrated that a feeder layer was not necessary and that growth could be initiated from single cells in a suitably constituted medium (*13*). The medium described in Table 1, when supplemented with serine alone, or with all the nonessential amino acids, has been demonstrated to be satisfactory for this purpose (*31*).

To produce clones, suspended cells are counted in a hemocytometer, diluted to a suitable concentration, and then plated in petri dishes. The dishes are incubated in a humidified incubator at 37°C in air containing 5 percent carbon dioxide, and visible clones form within seven to ten days. At this time they may be stained and counted, or the cells in a single clone can be harvested and used to initiate a new mass culture.

Genetic Studies with Human Cell Cultures

The availability of serially propagated cultures and mutant cells derived from them would suggest the possibility that a genetic analysis of human cells could be made, in the hope that transformation and genetic recombination could be demonstrated and that individual genes could be assigned to linkage groups and the order of genes on a single chromosome established. However, those cultures which have been maintained in an actively growing state for several years have all been shown to be aneuploid (*32*). While these aneuploid cultures exhibit a principal karyotype, they do contain a significant percentage of cells with other karyotypes. Further, mutants selected from a parent culture for drug resistance, morphological differences, or the ability to grow in a more limited serum-containing medium gave rise to cultures with karyotypes distinct from the parent culture—a finding which suggests that phenotypic variability of aneuploid cell populations may result from changes in

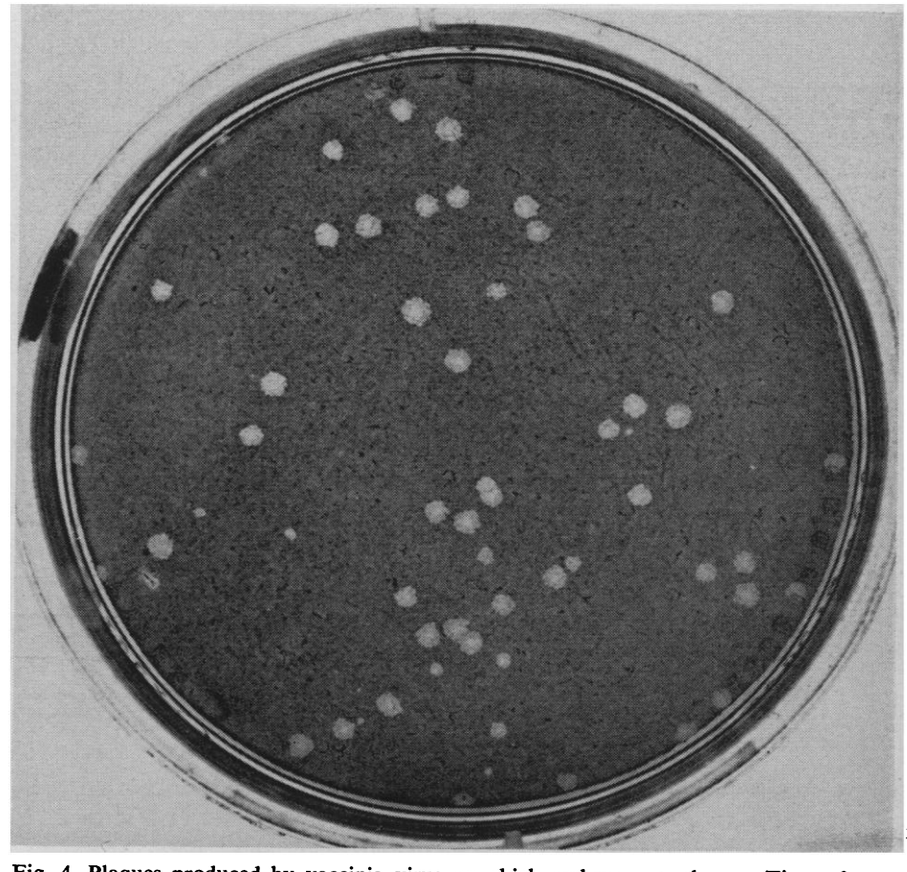

Fig. 4. Plaques produced by vaccinia virus on chick embryo monolayers. Tissue from ten-day-old embryos was used to prepare the monolayers, which were used 48 hours later. The dish shown above is stained with neutral red; the plaques are four days old.

the chromosomal constitution of the cells (*33*). In view of these drawbacks of aneuploid cultures, and since it has recently been shown that euploid cultures can be established and maintained under conditions of rapid growth for prolonged periods (*21*), these cultures would seem better suited for such experiments.

Euploid cultures initiated with tissue from skin biopsies of galactosemic individuals have been found to exhibit the characteristic metabolic lesion (*34*). The inability of these cells to grow in a galactose-containing medium, in contrast with the normal or heterozygous cultures, should make detection of transformed galactosemic cells possible, even if such transformation occurs with low efficiency.

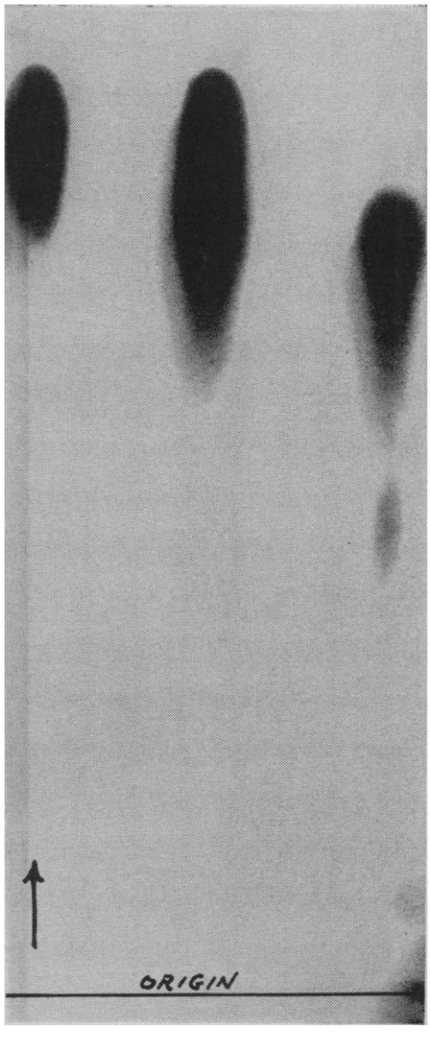

Fig. 5. A chromatogram of antitumor substances. The location of the active materials is detected through the use of KB cells in agar. The left band contains pluramycin (100 μg); the middle band, pluramycin (100 μg) and quinocycline (100 μg); the right band, quinicycline (100 μg). Descending chromatography was carried out in an ethanol, acetic acid, water (5:1:19) solvent system. [Courtesy of George B. Whitfield, Upjohn Company]

With the finding, by Tjio and Levan (*35*), that in human lung fibroblast cultures established from aborted embryos the true chromosome number of somatic tissue was 46 rather than 48 (Fig. 2), there was renewed interest in cytological observations on human cells. Since the report of Tjio and Levan, many additional observations on the human chromosome complement have been made. These studies have employed both short-term bone-marrow cell cultures and serially propagable fibroblast cultures established from solid tissue biopsies. While observations obtained by the two procedures have been similar, the procedure involving serially propagated cells has the potential advantage of permitting biochemical studies which may be related to the chromosome karyotype. To date there has been almost universal confirmation of the finding that the normal chromosome number in man is 46 and, further, that homologous autosomes from cells of different individuals are not significantly different in length or in the position of the centromere (Fig. 3). This close agreement in the results from several laboratories, indicating that the chromosome complement is identical in different normal individuals, has encouraged workers to study chromosomes in cases where there was prior indication that genetic abnormalities might exist.

Persons with chromatin-negative Turner's syndrome have been found to lack one sex chromosome, having only 45 (*36, 37*). In such patients, who are phenotypically female, no testes are present, the "gonads" consist of slender streaks of connective tissue, most secondary sex characteristics fail to develop at puberty, and other characteristic abnormalities are present. Males with chromatin-positive Klinefelter's syndrome—a condition in which spermatogenesis is scanty or lacking, the testes are small, and certain female traits are present—have 47 chromosomes, with an XXY complement (*38*). Females with the XXX complement, analogous to the "superfemale" *Drosophila,* have also recently been reported (*39*). These studies have established that in man, unlike findings for *Drosophila,* the Y chromosome is an important determinant of maleness, and that the XO and XY complexes are not equivalent.

An examination of the chromosome complement in cultures from patients with mongolism has revealed an extra small acrocentric autosomal chromosome (*40*), thought to arise as a result of nondisjunction during oogenesis. It must be noted that a variety of inherited conditions which have been examined have revealed a normal chromosome complement [for example, phenylketonuria and Gaucher's disease (*37*)].

Animal Viruses

The animal virus–animal cell system is presently being explored with many of the techniques which had previously been applied to the bacteriophage–bacteria system. While there are many similarities, the differences between the bacterial and mammalian systems indicate that qualitatively different observavations will result from a study of animal viruses. The occurrence of both ribonucleic acid–containing and deoxyribonucleic acid–containing animal viruses, the widely varied responses of cells to virus infection, and the possibility of determining the role of the subcellular structural components in the various stages of virus replication are some of the factors which make this an extremely attractive area of investigation. Viruses capable of eliciting tumor production in animals have been detected by means of cell cultures, and the mechanisms of the malignant transformation of cell cultures by viruses are under investigation (*41*).

In general, cell cultures have proved a powerful tool in the detection of a wide variety of previously unrecognized viruses. Light and electron microscopy and fluorescent antibody and radioautographic techniques make possible direct observations of intracellular viral development. Further, precise quantitation can be achieved by the use of the plaque assay system (*42*) (Fig. 4). The results of the many studies with animal viruses are described in recent reviews (*43*).

Cytotoxicity and Detection of Tumor-Inhibitory Substances

The effects of a number of compounds known to have antitumor activity in animals or man have been studied in cell cultures. A good correlation between cytotoxicity and anti-

tumor activity was demonstrated, and the results suggested the usefulness of cell cultures as a primary screen in the detection of antitumor agents (*44*). Procedures which depend on cytotoxic effects on animal cells have been developed for the isolation of such compounds from fermentation beers. The bioautographic and disk assay procedures, with animal cells embedded in or growing on agar, furnish methods for the rapid quantitation of the cytotoxic agents and for their identification (*45*). In the bioautographic technique, beers or other solutions are chromatographed, and the paper chromatogram is then placed in contact with agar previously seeded with cells. The diffusion of cytotoxic material from the paper and the resultant cell killing are detected with an appropriate viable cell strain. A typical bioautograph is shown in Fig. 5. The relative mobility of a cytotoxic agent in several different solvent systems aids in its characterization.

Similar techniques have proved of great value in the isolation of a large number of antibiotics from fermentation beers, and it is likely that growth-inhibitory materials highly active against animal cells will also be obtained. The examination of these compounds with respect to their antitumor activity in man will be of great interest.

The foregoing discussion illustrates the broad range of problems in which animal cell cultures are currently being used. While the papers from a single laboratory often tend to stress the importance of particular procedures in use in that laboratory, there is no evidence that the minor variations from laboratory to laboratory are of great importance, or that complex procedures are indeed more effective than the more direct one. It seems likely that new and improved cell-culture techniques will be forthcoming, in view of the larger number of investigators who are presently using cell cultures. For example, most studies with animal cell cultures have, until recently, utilized aneuploid cells. Procedures which have recently been described permit the establishment and maintenance of rapidly growing euploid cultures (*21*), and it seems likely that these cultures will now be more widely used. Euploid cultures have yielded important genetic information and undoubtedly will continue to prove of great value in this area. Further, they may help us understand what factors determine whether cells in culture continue to carry out the specialized functions associated with tissues in vivo. The establishment of serially propagated, functioning animal cell cultures will open up broad new fields of wide significance to scientists in many disciplines.

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