USE OF CELL CULTURE IN PHARMACOLOGY[1,2]

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During the last 15 years, cultures of cells *in vitro*, because of the introduction of quantitative methods of high precision, have become a valuable tool for studies of the effects of certain classes of pharmacological agents. Cell cultures offer the possibility of observing the effects of drugs on cells in an artificial environment, without interference by nervous and humoral factors present in the intact organism. Furthermore, because during preparation of primary cultures the cells are released from the three-dimensional framework of tissue architecture, the influence of intercellular contact is greatly decreased or completely abolished. Cell culture systems thus permit an analysis of the responses of isolated cells to pharmacological agents. In this review, various applications of cell culture methods to the analysis of the mode of action of drugs are discussed. The action of certain drugs is characterized by a high specificity, and inhibition of a single process within the cultured cells is observed. Such specific inhibitors have become extensively used in analyzing the role of various processes in cellular activities. This "use of pharmacology in cell culture" will, however, not be considered.

After a primary culture of dispersed cells has been prepared from a piece of tissue by mechanical disruption or by the application of a suitable enzyme such as trypsin, in most cases only a small proportion of the cell population is capable of proliferation. Furthermore, under conditions *in vitro* the cells usually undergo marked changes of their metabolic activities. Even a primary culture, therefore, cannot be regarded as representative of the tissue of origin. Under cell culture conditions, in general a selection of cell types capable of continued proliferation takes place. In contrast to the situation in the intact organism, cell multiplication *in vitro* is not limited by growth-regulating mechanisms. Cells in prolonged culture, therefore, are, in some respects, comparable to multiplying microorganisms. Genetically pure cell populations may be obtained by culture of isolated cells. Normal human cells in culture usually retain their diploid chromosome set. They appear, however, to be limited in their proliferative capacity to a number of approximately 60 cell divisions and then undergo degenerative changes which

[1] The survey of literature pertaining to this review was concluded in April 1968.

[2] The following abbreviations are used: FUdR (floxuridine; 5-fluoro-2′-deoxyuridine), BUdR (5-bromo-2′-deoxyuridine), IUdR (idoxuridine; 5-iodo-2′-deoxyuridine), ara-C (cytarabine; 1-β-D-arabinofuranosyl-cytosine).

ultimately lead to the loss of the culture. Cells of the mouse and of many other species, on the other hand, after a certain number of cell generations in culture, undergo a transformation which expresses itself in morphological alterations, changes in chromosomal constitution and the ability to multiply indefinitely *in vitro*. Frequently transformed cells grow as malignant tumors upon reinjection into histocompatible animals.

Methods of Cell Culture

Mammalian cells may be cultured either as monolayers attached to a solid substrate, or as cell suspensions. Certain cell types always form monolayers and cannot be cultured as suspensions, whereas the cells of other lines remain in suspension if appropriately agitated (spinner cultures). Some further cell types do not attach to the surface of culture vessels even in the absence of any agitation. In monolayer cultures, exchange of the medium by a different one is very easy. Passage of monolayer cultures, however, requires the use of trypsin, versene or another agent which dissociates the cells from the solid substrate. Suspension cultures, on the other hand, offer the possibility of withdrawing aliquots of the cell population at suitable time intervals. In addition, they can be kept indefinitely in the exponential phase of growth by simple dilution with fresh medium.

For most cell lines, a medium containing minerals, glucose, amino acids, vitamins and a small amount of dialyzed serum has proved to be satisfactory (1). This type of semisynthetic medium, because all of its low-molecular weight components are known, provides for reasonably reproducible culture conditions and has been used with good success to study the effects of antimetabolites which depend on the concentration of certain normal nutrients or metabolites. Completely defined media containing no serum proteins do not support the reproduction of most mammalian cells. Various cell lines, however, have been adapted to such defined media by selection of nutritionally less demanding cells.

Several methods for determination of cell number and cell multiplication have been developed. Cells may be counted microscopically or by electronic devices such as the Coulter counter. Cell multiplication may also be estimated by measuring the increase of specific cell components, such as DNA, total nucleic acids, proteins and others. In order to assess cell damage due to the effects of pharmacological agents, various viability tests, such as measurement of the capacity of cells to exclude certain dyes, may be applied. The impairment of the capacity of cells to multiply may be determined by counting the colonies developing from a given number of isolated cells on a solid substrate (2) or suspended in a semisolid medium (3).

The reproducibility of cell culture experiments is greatly increased because of the possibility of freezing viable cells for prolonged periods of time. By this technique, changes of cellular characteristics due to selection of mutant cell types by the culture conditions are avoided and a supply of cell populations with identical properties is provided. Furthermore, the dan-

ger of infection of cultures by viruses or other microorganisms, such as mycoplasmas, is considerably diminished. The homogeneity of the response of cell cultures to pharmacological agents may be further enhanced by using synchronizing techniques. Synchronously dividing cultures are obtained by treatment with certain inhibitors or by separating cells which are in one phase of the division cycle from the remainder of the cell population. The action of drugs may thus be analyzed on cells which are in a specific phase of the division cycle.

Cytostatic Agents

Evaluation of potential antineoplastic drugs.—Cultures of neoplastic cells represent an attractive model of malignant tumors. In contrast to the growth of tumors *in vivo,* cellular reproduction in culture can be measured easily and accurately. Therefore, the possibility of using *in vitro* cultures of neoplastic cells for the assessment of carcinostatic activity has attracted much attention. Surprisingly, cultures derived from normal tissues were found not to differ significantly from cultures of neoplastic origin with respect to their susceptibility to carcinostatic agents (4, 5). The high sensitivity of cultures of normal cells probably is due to their high rate of cell proliferation. In fact, it is well known that rapidly multiplying normal cells *in vivo,* such as cells of hemopoietic tissues or of the gastrointestinal mucosa, also exhibit a high sensitivity to most antineoplastic drugs in use today. Normal cells in culture apparently do not acquire their high sensitivity to cytostatic treatment as a result of a gradual change of characteristics toward those of neoplastic cells: primary cultures of normal and neoplastic cells were also shown to be equally sensitive to a number of cytostatic compounds (6). Cell cultures, therefore, cannot be used to detect differences in toxicity of a given compound to normal and cancer cells, and no definite conclusions on the therapeutic efficacy of a cytostatic substance may be drawn from results obtained with cell cultures.

On the other hand, the results of several studies indicate that substances with antineoplastic activity *in vivo* usually are characterized by a rather high toxicity for cell cultures (4, 5, 7). There is, however, no good correlation between cytotoxicity *in vitro* and antineoplastic activity *in vivo*. Such a correlation apparently is restricted to groups of compounds chemically related to each other, such as folic acid antagonists (4) or corticosteroids (8). Cell cultures, therefore, are of limited usefulness in screening for drugs with antineoplastic activity. They may be used in a primary screen to detect agents with possible carcinostatic activity. There will, however, always be a certain proportion of "false positives" and "false negatives". While false positives can be recognized in a second test system, e.g., by using transplantable tumors in experimental animals, false negatives will be lost for subsequent studies if cell cultures are used as the exclusive primary screen. For more restricted purposes, however, cell cultures are of considerable value. For instance, they may be used to follow the purification of anti-

biotics with cytotoxic activity from culture broths (9). Furthermore, in groups of compounds with similar chemical structure, cell culture systems may allow detection of the most active substance.

Another possible application of cell culture methods is concerned with the choice of the drug which is most promising in a particular patient with neoplastic disease. For instance, short-term cultures of leukocytes from patients with leukemia or leukolymphosarcoma were used to study the inhibition by various chemotherapeutic agents of uridine incorporation into RNA. A good correlation between the effectiveness of corticosteroids and vincristine *in vitro* and the clinical response to treatment with the respective drug was observed (10). For the prediction of therapeutic efficacy in individual patients, not only cell cultures but also slices of tumor tissue may be used (11). Biochemical tests apparently provide more satisfactory criteria than measurements of cell reproduction.

Carcinostatic drugs which have no inhibitory effects on neoplastic cells *in vitro* constitute the false negatives in a cell culture screening system. Such substances in general acquire their carcinostatic activity only after being converted by metabolic processes *in vivo*. For instance, 6-azauracil, although active against transplantable tumors, does not inhibit reproduction of neoplastic cells in culture. 6-Azauridine, however, is inhibitory also to cultured cells (12). Cell cultures are characterized by a relative inability to utilize free pyrimidines (13), whereas natural pyrimidines, as well as 6-azauracil, are converted to ribonucleosides in certain normal cells, such as in liver, of the intact organism (14). Pyrimidine ribonucleosides, on the other hand, are efficiently incorporated into the nucleotide pool of cultured cells (13). Thus 6-azauridine is transformed to 6-azauridylic acid, an inhibitor of orotate decarboxylase (15). Similarly, 5-fluorouracil riboside and deoxyriboside were found to be much more active as inhibitors of cell multiplication than 5-fluorouracil (16).

Cytoxan represents another example of a drug which exerts a carcinostatic activity *in vivo,* but does not inhibit growth of cell cultures. This difference is due to the inability of cultured cells to convert the drug to a metabolite with the characteristics of a biological alkylating agent, whereas liver and certain other tissues are capable of carrying out this metabolic activation (17, 18).

Structure-activity relationships.—Although cell cultures are of limited value in the assessment of antineoplastic activity, they have proved to be useful in studies of the relation between chemical structure and biological activity of cytostatic drugs. Activities observed in cell culture are independent of the effects of absorption, distribution and excretion of a drug in the intact organism, and usually metabolic alterations are very limited as compared to conditions *in vivo*.

In a series of six folic acid antagonists, aminopterin exhibited the highest inhibitory activity on several cell culture lines tested (4). The activity of metabolic antagonists may also be determined on the target enzyme in a

cell-free system. Cell cultures, however, are of special value in studies of drugs whose mechanism of action at the molecular level is not known. They have been used with good success to determine the activity of various colchicine derivatives. It was shown that the methoxy group of the tropolone ring may be replaced by an amino group (19), or the acetyl group of the acetylamino side chain by a methyl group (20) without appreciable change in activity. On the other hand, removal of the entire acetylamino side chain resulted in a ten-fold increase of activity (21). Similarly, various steroids were compared with respect to their capacity to inhibit cell reproduction in culture. A considerable variation of the response of different cell lines to individual steroids was observed (22). Lines derived from lymphocytic leukemias apparently are characterized by a relatively high sensitivity to glucocorticosteroids. Steroids which effectively suppressed cell multiplication in leukemic cell cultures also were highly active as inhibitors of leukemic cell proliferation in mice (8) and as anti-inflammatory agents (23). More recently, the capacity of several hydroxyurea derivatives to inhibit DNA synthesis of cell cultures was compared. N-methyl hydroxyurea was found to be somewhat more active than hydroxyurea itself (24).

Mechanism of action of cytostatic agents.—The use of cell culture methods has resulted in important contributions to present knowledge of the mechanism of action of cytostatic substances. Homogeneous populations of cultured cells may be exposed to an inhibitor during variable time intervals and in a defined environment, and the concentration of the inhibitor to be tested can be kept constant without technical difficulties. Certain conclusions as to the mechanism of action may sometimes be drawn from the kinetics of inhibition and from the relationship between concentration of the inhibitor and the observed effects. In addition, differences between the mode of action of related drugs may be detected by this approach.

Further analysis of the mechanism of action may be based on the observation of metabolic changes subsequent to the addition of an inhibitor to cell cultures. For instance, preferential inhibition of one type of macromolecular synthesis may be detected. Similarly, morphologic alterations frequently afford valuable clues as to the mechanism of action. Thus, chromosomal damage after treatment with biological alkylating agents is suggestive of preferential damage to DNA, while an accumulation of metaphases results from treatment with spindle poisons, such as colchicine, *Vinca* and *Podophyllum* alkaloids. A metabolic block in a biosynthetic chain may be localized more precisely by comparing the incorporation rates of various precursors. A precursor which is fed into the biosynthetic chain prior to the block will show a decreased incorporation in the presence of the inhibitor, while a precursor which circumvents the block will be incorporated more effectively under these conditions.

An unequivocal identification of the metabolic alteration that is responsible for the inhibition of cell reproduction is not possible on the basis of biochemical studies alone. On the other hand, definitive

the site of attack of a cytostatic agent may sometimes be drawn from an analysis of antagonisms between inhibitors and normal nutrients or metabolites. Similarly, use of a second agent to modify certain cellular responses may contribute to the elucidation of mechanisms of action of drugs. Thus, alteration of sensitivity to an inhibitor after incorporation of an analogue into DNA suggests that the primary site of attack of the inhibitor is situated on DNA. Further information as to the mode of action of cytostatic agents may be obtained by observing their effects on cells in different phases of the cell cycle. In the following, these possibilities will be illustrated with selected examples of antineoplastic drugs.

5-Fluoro-2′-deoxyuridine (FUdR) was found to cause a rapid inhibition of DNA synthesis in cultured cells, while RNA and protein synthesis were not affected, at least for a certain time (25, 26). In the presence of FUdR, the incorporation of formate-^{14}C (26) or orotate-^{14}C (16) into DNA was decreased, whereas the incorporation of labeled thymidine was increased (16). Inhibition of cell multiplication by FUdR was prevented by thymidine independently of the concentration of the inhibitor. On the other hand, reversal of the inhibition by uridine depended on the concentration of FUdR (16). These results support the assumption that inhibition of cell reproduction by this inhibitor (or a metabolite thereof) is due to a block in the synthesis of thymidylic acid from deoxyuridylic acid. In fact, thymidylate synthetase from Ehrlich ascites carcinoma cells was reported to be inhibited by FUdR-monophosphate (27).

1-β-D-Arabinofuranosyl-cytosine (ara-C) markedly inhibits DNA synthesis of cell cultures while accumulation of RNA and protein are not significantly affected in short-term experiments (28, 29). The inhibition of cell multiplication by ara-C was prevented by deoxycytidine, whereas cytidine, deoxyuridine or thymidine did not exert this protective effect (28, 30). Furthermore, incorporation of uridine-^{3}H into deoxycytidine phosphates, but not into cytidine phosphates, was reduced in the presence of ara-C (30). Low concentrations of ara-C were found to inhibit the incorporation of cytidine and thymidine into DNA, while deoxycytidine was utilized more efficiently for DNA synthesis under these conditions. Reduction of cytidine ribonucleotides to the corresponding deoxyribonucleotides, however, was not inhibited by ara-C unless small amounts of deoxycytidine were present in the medium (31).

Hydroxyurea also causes a rapid inhibition of DNA synthesis without concomitant inhibition of RNA and protein synthesis (32, 33). Cultured Chinese hamster cells synthesizing DNA at the time of addition of the inhibitor were irreversibly damaged within a short time, while cells in other stages of the division cycle were prevented from beginning DNA synthesis, but remained viable for several hours (34). HeLa cells, on the other hand, were not lethally damaged during prolonged exposure to the drug. No effect on cells in G_1 and G_2 periods of the cell cycle was observed, whereas DNA

synthesis of cells in the S period was reversibly inhibited (35). The inhibition of DNA synthesis by hydroxyurea in L-strain cell cultures was prevented by the addition of the deoxyribonucleosides of adenine, guanine, cytosine and thymine to the culture medium (36). This protective effect of added deoxyribonucleosides, however, was incomplete in HeLa cell cultures (24, 37) and could not be confirmed with cultures of a hamster cell line (33).

Pyrimidine analogues in which the hydrogen atom in position 5 is replaced by halogen, exhibit many common properties with natural pyrimidines. 5-Bromodeoxyuridine (BUdR) and 5-iododeoxyuridine (IDuR) are incorporated in place of thymidine into DNA of mammalian cells in culture (38). If the introduction of halogenated uridine into DNA does not exceed a certain extent, cellular viability and capacity to multiply are maintained (38–40). The toxicity of IUdR is higher than that of BUdR, since less IUdR than BUdR may be incorporated before loss of cellular viability occurs (40). BUdR and IUdR share the specificity of thymidine as precursors of DNA. Incubation of cell cultures with these analogues, therefore, results in chemical modification of DNA without alteration of other cell components. Changes of cellular characteristics observed following incorporation of these halogenated pyrimidines are hence attributable to changes in DNA. Incorporation of BUdR and IUdR were shown to cause a markedly increased sensitivity of cells to ionizing and ultraviolet radiation (40–42). From these findings it was concluded that damage to DNA is responsible for reproductive cell death observed after ionizing and ultraviolet irradiation.

Biological alkylating agents react with many different cellular constituents, and after treatment with these cytostatic drugs, a considerable number of biochemical changes may be observed. Many experimental observations support the assumption that chemical modification of DNA is responsible for the effect of alkylating agents on the capacity of cells to multiply. After exposure of human cells in culture to mitomycin C, covalent crosslinking of complementary DNA strands was demonstrated (43). Incorporation of BUdR into cellular DNA resulted in an increased sensitivity of murine neoplastic cell cultures to nitrogen mustard and 2,5-hexanediol dimethanesulfonate (Dimethyl myleran) (44). In cell cultures treated with biological alkylating agents, DNA synthesis is inhibited to a greater extent than RNA or protein synthesis (45–47). This differential inhibition causes cell enlargement, and giant cells may develop (48). By means of autoradiographic methods it was shown that one of the main effects of treatment of cell cultures with nitrogen mustard consists in an arrest of cells in the period of DNA synthesis of the division cycle. Cells in other phases of the cell cycle usually are blocked only when they reach the S period. DNA of cells blocked in the S period may be synthesized in amounts up to four to six times greater than normal, thus corresponding to several cycles of replication (49, 50). Cells which escape from the block in the S period are definitely arrested in

metaphase. Although the effects of different alkylating agents are, in many respects, strikingly similar, differences between the mode of action of nitrogen mustard and that of Dimethyl myleran have been described (44, 51). After incubation of cell cultures with labeled sulfur mustard, a release of labeled material from the cellular DNA upon further incubation of the cells was observed. This release appeared to be mediated by an enzyme and may be related to recovery processes (52). In cell cultures treated with various inhibitors of DNA synthesis, RNA and protein synthesis usually continue despite the interruption of DNA synthesis. This metabolic imbalance results in an increase in the cellular RNA and protein content with concomitant increase in cell volume (53).

Dactinomycin (actinomycin D) causes an inhibition of cellular RNA synthesis at concentrations which do not affect DNA or protein synthesis (54). The nuclear synthesis of all classes of RNA is inhibited by actinomycin D; the attachment of terminal nucleotides to soluble RNA, however, is not modified (55). Treatment of cell cultures with low concentrations of actinomycin D selectively blocks nucleolar RNA synthesis and results in a preferential inhibition of ribosomal RNA synthesis (56, 57). In HeLa cell cultures treated with actinomycin D, cytoplasmic enlargement and a marked increase in the number of mitochondria was observed (58).

Mechanisms of resistance to cytostatic agents.—Cell cultures represent a convenient model system for the analysis of the development of resistance to anticancer agents. Cellular resistance to antineoplastic drugs has been shown to be due to a heritable change of biochemical characteristics. Resistance usually is maintained in the absence of the inhibitor during many cell generations. Evidence for the genetic basis of resistance was obtained in transformation experiments with isolated DNA. Thus, resistance to 8-azaguanine was induced in drug-sensitive cell cultures with DNA of drug-resistant cells (59). Conversely, azahypoxanthine-resistant cells were transformed by DNA from azahypoxanthine-sensitive cells (60, 61). In mixed cultures of cells resistant to 8-azaguanine and to BUdR, formation of hybrid cells sensitive to both inhibitors was demonstrated (62, 63). As shown earlier for bacteria, a more detailed analysis revealed that mutation to drug resistance in cell cultures may occur in the absence of the inhibitor. The drug, therefore, in general does not induce mutations, but provides for selective conditions favoring multiplication of the resistant mutant cells. Mutation rates of the order of 10^{-3} to 10^{-6} mutations per cell, per cell generation, have been reported (64, 65). Resistance to certain drugs, such as 8-azaguanine, develops in one or a few steps (66–68), whereas resistance to other inhibitors, such as puromycin and methotrexate (amethopterin) evolves in many small steps (66, 67, 69).

For biochemical studies of mechanisms of resistance, transplantable tumors as well as cell cultures may be used. The latter, because of the homogeneity of their cell populations, offer certain advantages. The cells of mutant sublines in culture resistant to methotrexate were found to contain

greatly increased amounts of the target enzyme, folic acid reductase (70, 71). The increase in enzyme activity apparently was not due to qualitative differences of enzyme structure, but to an enhanced synthesis of folic acid reductase (71, 72). A second mechanism of resistance to methotrexate is based on a decreased uptake of the inhibitor by the cells (73). Resistance to purine analogues usually is associated with the decrease or loss of the enzyme responsible for conversion of the analogue to the corresponding ribonucleotide. Cell lines resistant to 6-mercaptopurine or 8-azaguanine were found to be defective in the enzyme forming the ribonucleotide of these analogues and of hypoxanthine and guanine (74–77). A more detailed analysis with hyperdiploid L-strain cells revealed that resistance to 8-azaguanine and decrease in cellular guanylic-inosinic acid pyrophosphorylase developed in three distinct steps, possibly related to inactivation of three gene loci responsible for synthesis of this enzyme (78). Resistance to 2,6-diamino-purine, on the other hand, was reported to be associated with a deficiency of the enzyme converting this analogue, as well as adenine, to ribonucleotides (74, 79). Similarly, cells resistant to pyrimidine analogues were reported to be defective in the enzyme responsible for conversion of the nucleoside to the corresponding nucleotide. Resistance to FUdR or BUdR, for instance, may be accompanied by loss of thymidine kinase. Other mutant cell lines resistant to BUdR, however, are characterized by continued incorporation of the analogue into cellular DNA, with maintained capacity of cellular reproduction (80, 81).

Elucidation of mechanisms of resistance to carcinostatic agents often permits certain conclusions as to the mechanism of action as well. For instance, the findings of an association between resistance to a drug and a specific enzyme deficiency support the assumption that the drug has to be converted by the cellular metabolism in order to exert its inhibitory activity.

Antiviral Agents

In contrast to their use in the assessment of antineoplastic activity, cell cultures represent a valuable test object for screening of antiviral agents. In virus-infected cell cultures the effects of drugs on both the host cell and the virus may be studied. Therefore, preliminary conclusions on the therapeutic efficacy may be drawn. For the evaluation of the effectiveness of antiviral drugs different criteria are available, such as survival time of infected cultures, reduction of virus yields, inhibition of cytopathic changes, inhibition of plaque development in infected monolayers, or complete cure of cultures. Beside their use in screening for antiviral agents, cell cultures have been of value in the elucidation of mechanisms of action of antiviral drugs and of specific processes during the infective cycle of viruses (82). This is, in part, due to the possibility of synchronous infection of a cell population with the free choice of the number of infectious particles per cell.

Guanidine and 2-(α-hydroxybenzyl)-benzimidazole prevent the appearance of viral RNA polymerase of susceptible picornaviruses, but have no

effect on the function of the enzyme once it has been formed (83, 84). The precise sites of inhibition of these two drugs, however, are probably not identical. Methisazone (isatin-β-thiosemicarbazone) inhibits the formation of certain proteins required for maturation of pox viruses, while synthesis of viral DNA and of proteins required early in the reproductive cycle is not affected (85). This effect appears to be mediated through a markedly reduced life span of messenger RNA responsible for synthesis of "late" proteins (86). IUdR is capable of eliminating herpes simplex and vaccinia viruses from infected cell cultures even if the infection has already produced cytopathic changes (87). IUdR is incorporated into viral DNA in pseudo-rabies-infected cells to a much greater extent than into cellular DNA of control cells (88). Apparently as a consequence of formation of IUdR-containing viral DNA, nonfunctional viral proteins are formed and assembly into viral particles is impaired (89). Ara-C, on the other hand, inhibits replication of DNA of herpes simplex virus (90). The inhibition of DNA synthesis by ara-C, however, was less pronounced in cell cultures infected by herpes viruses than in noninfected control cultures (91). Amantadine was shown to inhibit penetration, but not adsorption of susceptible viruses to cells in culture (92).

Carcinogenic Chemicals

Various carcinogenic chemicals are capable of inducing transformation of hamster and mouse cells in culture. Under appropriate conditions, the transformation occurs in a high proportion of the cell population and expresses itself in hereditary changes such as a decrease of contact inhibition, a random pattern of growth, and the ability to grow as malignant tumors upon reinjection into histocompatible animals. Several chemically related, but noncarcinogenic substances did not exert these effects (93, 94). While multiplication of normal rodent cells in culture is inhibited by treatment with different carcinogenic chemicals, neoplastic cells of these species in general were found to be much more resistant to the toxic action of such compounds (95–97). In cultures of human origin, however, no difference in susceptibility between normal and neoplastic cells was observed (97).

Agents which Modify Specific Functions of Differentiated Cells

Various differentiated cells may be grown in culture without loss of their specific functions. This offers the possibility of studying modification of these functions by drugs under culture conditions. For instance, beating heart cells in culture were subjected to the action of substances such as cardiac glycosides, adrenergic stimulating and blocking agents, quinidine and steroids (98, 99). Furthermore, the morphological and biochemical effects of thyrotropin on thyroid cell cultures were investigated.

The modification by pharmacological agents of specific functions of connective tissue cells in culture has been dealt with in numerous studies. In cultures of connective tissue origin, production of acid mucopolysaccharides

and of collagen may be observed. Collagen production by cultured human fibroblasts was found to be highly dependent on the presence of ascorbic acid in the medium, while hyaluronic acid synthesis occurred also in the absence of ascrobic acid (100, 101). 17-β-estradiol elicited an increase in acid mucopolysaccharide production by human embryonic fibroblasts. The multiplication rate of the cells, however, was depressed by this treatment (102). Hydrocortisone caused a decrease in collagen and mucopolysaccharide formation (103, 104), and the intrinsic viscosity of hyaluronic acid produced during hydrocortisone treatment was reduced (105). Several antiinflammatory drugs were found to depress glucosamine incorporation into acid mucopolysaccharides of fibroblast cultures at concentrations that did not affect cell proliferation (106).

Conclusions

Due to the introduction of cell culture methods, important contributions to the pharmacology of drugs which modify proliferative processes have been possible. Thus, studies on cultures *in vitro* have led to a better understanding of the mode of action of many cytostatic agents and of the mechanisms underlying resistance of cells to these drugs. Furthermore, detection of the activity of antiviral compounds and analysis of their mechanism of action have benefitted to a considerable extent from the application of cell culture methods.

Cell cultures have also been used to study the action of carcinogenic chemicals. Up to now, however, the results have been mainly descriptive, and it remains to be seen if the mechanisms of chemical carcinogenesis may be elucidated by analyzing the effects on cell populations *in vitro*. The actions of drugs affecting certain specific functions of differentiated cells have also been studied in cell culture. However, the interpretation of results obtained with dispersed cell populations appears, in most cases, rather difficult.

Cell cultures apparently represent a useful tool in studies of drugs whose effects can be recognized at the cellular level. In comparison with more complex test systems, cell cultures offer several advantages, such as free access of the drug to the cells, lack of changes in drug concentration due to excretion mechanisms, homogeneity of the cell population and a strictly controlled but variable environment of the cells.

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