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1. General Considerations

In the last few years, an increasing number of viruses have been identified which are implicated in the development of tumours. It cannot be assumed that their action in all cases is necessarily direct, and even if it were, then their oncogenic effect may be dependent on, or modified by, other factors such as genetic resistance or hormone levels.

Whilst no human cancer has yet been shown to be caused by a virus, it is difficult to believe that man is an exception to the widespread phenomenon of virus-induction of tumours seen in other mammals, amphibia and birds. Experimentally, human viruses have been shown to be capable of provoking cancers in animals. Also, human cells grown in vitro have been shown to undergo "transformation" after infection by oncogenic viruses.

On this indirect evidence it seems probable that at least some human tumours may be shown to be dependent in some way on a virus infection. From experimental work on animals it is clear that some oncogenic viruses may lie dormant within the body for a period extending over an appreciable part of a lifetime. At a later stage the virus may be activated through exposure of the host cells to certain chemicals or radiation; in many respects the position resembles that of lysogeny in bacteria although there are important differences.

Whilst the behaviour of a virus as an oncogenic agent differs considerably from that of a virus in the common infectious diseases, nevertheless, an antiviral substance effective in treatment of the latter may also prove effective at some stage in the treatment of a virus-induced tumour.

Apart from the question of a common type of aetiological agent, the design of a screening programme for active antiviral agents resembles fairly closely that for anti-cancer substances. Again, the source material for both in a random screen will be similar. In the case of both cancer (whether induced by virus or not) and straightforward viral infection, there are excellent reasons for seeking agents which selectively inhibit the replication or function of particular types or portions of nucleic acid within cells. POTTER (1964) has discussed comprehensively how the development of cancer cells might be controlled through regulation of gene expression.

No attempt is made here to cover the synthetic drugs formulated for possible activity in the treatment of either cancer or viral diseases; the purpose is to consider only those agents derived from natural sources. It may well be that in due course, some of the naturally occurring drugs may be synthesised or modified and possibly improved on by the introduction of more effective analogues; in another field the extension of the penicillin range of antibiotics is an example of this kind of development.

Intrinsically linked with the search for new antiviral and antitumour agents is the choice of suitable screening systems. For the former this lies plainly between the use of

in vivo and in vitro tests. It can be argued that the in vivo tests save considerable time and effort since compounds which fail to produce an effect in these conditions will not be of clinical use; from this point of view, preliminary in vitro study of such agents is wasted effort. On the other hand, in vitro tests are both less expensive and more convenient for the screening of large numbers of test substances. Inevitably, many agents show positive results when tried in vitro, but prove subsequently ineffective or otherwise unsuitable in animal experiments. A substantial wastage rate is to be expected from the use of a preliminary in vitro screen therefore.

To a large extent, these same considerations apply to the testing of anticancer drugs. However, it can be argued that the use of in vitro tests may be very misleading since the behaviour of cells grown in culture over numerous cell generations is likely to be different from that of the original source from which the line was established. In spite of this, there is a fair correlation between the degree of cytotoxicity seen in these conditions and the useful activity against tumours in intact animals.

The use of animal neoplasms is not free from objections, however. There may be little resemblance between the response of an animal and a human tumour arising in the same type of organ, even if of similar histological appearance. Yet this objection can be put forward also when dealing with human tumours of similar histological type—some may respond well to a certain form of treatment and others show little or no response; the difficulties encountered in trying to correlate the histological appearance of breast cancers with the prospects of their response to hormone therapy is a case in point. Such a tumour needs individual assessment according to its particular metabolic pattern. It is not surprising therefore, that in surveying the results of clinical trials with some particular anticancer agent, only a proportion of a certain type of neoplasm may respond, yet a few sensitive tumours may be found amongst a wide variety of cancerous growths. Nevertheless, a drug which is effective against only a small percentage of tumours may yet be the drug of choice in some particular case.

Knock (1967) has argued skilfully and convincingly of the need for assessment of response of individual tumours to a range of anticancer agents at the time of operation. There is a two-fold purpose here; first, to ensure the most appropriate choice of drug for suppressing the growth; second, to avoid the use of individually-ineffective yet toxic agents which can do nothing to stem the further development of the particular cancer in question, but which depress the general condition of the patient still further. In some circumstances, it is possible that the drug therapy may also reduce or inactivate an immunological response which may at least have been restricting the growth in its progress (Hitchings and Elion, 1963).

Using a system for selection of chemotherapeutic agents against individual tumours, a wider range of such substances can be considered even though many may be active in only a small proportion of cases. In the absence of such method of assessment, the choice of drug will be governed by knowledge of the relative percentage of tumours of that type which respond; the higher the figure then the more likely is that choice. Here the range of drugs considered is likely to be much narrower, and an effective agent for an individual case may thus come to be overlooked.

The situation is not the same as with antibiotics in the treatment of bacterial infection. In the latter it is true that an assumption can be made on statistical evidence leading to the choice of treatment which is likely to be effective in dealing with a

particular organism. In some cases, however, the bacteria may be resistant and valuable time lost if a wrong drug is used, hence the use of sensitivity tests for individual infections. Other than the loss of time, which may yet be dangerous, there is no actual positive damage to the patient resulting from wrong choice of antibiotic since these agents are relatively non-toxic. The anticancer drugs are quite different in this respect since the doses used in treatment inevitably cause unpleasant side effects.

Considération of recent advances in the field of antiviral and anticancer therapy entails then, in addition, a brief review of screening procedures and of techniques for drug selection in clinical cases.

2. The Design of Screening Programmes

In vitro versus in vivo

The problem of testing is a theory one, even more so for antitumour than antiviral agents.

The first choice for both lies between in vitro and in vivo tests. Many agents which are effective against viruses or tumour cells in vitro are of no value in vivo; the wastage rate is high. Rapid inactivation and excessive toxicity are amongst the factors which underlie the failure of so many compounds in this respect. However, some agents which subsequently prove to be of no clinical use, may nevertheless be of academic value in the study of virus-cell interactions and metabolic pathways in vitro. A fuller knowledge of the relationship between interference with specific metabolic pathways and the manifest activity of a drug is likely to be of value in the development of more effective compounds.

In vitro tests have the great advantage of being relatively cheap, simple and appropriate for the rapid screening of large numbers of test substances. Those which show activity are in a minority and can be selected for more elaborate study in intact animals. Whilst the majority of compounds which are active in vivo are also active in vitro, nevertheless some agents would be overlooked if the primary screen is in vitro. There are two possible causes for this. One is that the agent itself is inactive but is metabolised in the body to an active form. The other is that it may act through stimulation of cellular defence mechanisms rather than in a direct manner. The method of Dice et al. (1965) is an ingenious way to avoid the former difficulty; these workers test in vitro for antiviral activity using serum obtained from animals after injection of the drug.

In assessing the value of active antiviral filtrates derived from soil isolates, Ehrlich et al. (1965) use a Virus Rating which is a measure of the extent of inhibition of viral cytopathic effect at levels which are non-toxic or not very toxic for the host cells. A V.R. of 1.0 or more was used as a basis for further testing. Of several thousand samples tested, about 0.1% were active against the four viruses of herpes simplex, parainfluenza-3, measles and poliovirus type 2.

Once activity has been detected it is essential to decide whether a substance is active enough to justify the cost and time of further testing. The best statistical approach to this problem is by the use of sequential procedures, and the subject has been discussed in a concise and masterly manner by Rosenoer (1966) in relation to

the testing of potential antitumour drugs. The criteria are selected on a basis which weighs the chance of missing an active agent against that of including one with little or no activity. Experimental tests should be carried out on known active and inactive agents to ensure that the criteria which have been adopted are satisfactory.

Antiviral Screens

While agents of the interferon class are active against a very wide range of viruses, there are some antiviral substances which act only against a small number. For instance, phagicin (CENTIFANTO, 1965) has a more restricted range of activity and is useful against vaccinia and herpes simplex viruses yet not against some RNA viruses. Ideally, a primary screen should include at least one representative of each major group of viruses; primary in vivo screens for viruses are in a minority at present. BAUER (1966) has discussed the question of possible correlation between the size of viruses and necessary concentrations of antiviral substances; the smaller the virus, the higher the concentration of antiviral agent necessary to inhibit replication.

There are several stages in the life cycle of viruses where an antiviral agent may act. In the first place, the virus is in a free state in the extracellular environment. This is followed by attachment to an appropriate cell—the type being dependent on the specificity of receptors on the cell surface and the antigenic structure of the virus. Penetration of the cell membrane succeeds attachment, and is itself followed by uncoating of the viral nucleic acid, and later by synthesis of enzymes. The next stage is synthesis of viral nucleic acid and protein through the action of the new enzymes. Then assembly of the viral components and maturation follow, with release finally of the new virus particles.

Clearly, antiviral agents which act only on the early stages of virus infections, such as attachment or penetration, can only be useful for prophylaxis. Amantadine, a synthetic compound, falls into this class through its action in blocking penetration of the cell membrane by the virus (HOFFMANN et al., 1965).

In many viral diseases the symptoms follow the main peak of viral synthesis, and in these cases there seems to be little real chance of alleviating the situation except by prophylaxis. However, there are two classes of viral infections which are now assuming importance, and which differ greatly from the picture of acute infectious viral disease. One is the group of "slow" viruses, and the other the oncogenic viruses. The extent of their participation in human disease is not known at present.

The virus of kuru has recently been isolated, and from the long course of this illness it seems possible that an effective antiviral agent might arrest it in its progress. Normally regarded as being responsible for an acute febrile illness, it now appears that measles virus is responsible for subacute sclerosing panencephalitis. Again, whilst the cause of a demyelinating disease such as disseminated sclerosis is obscure, it may also be of viral origin.

In regard to cancer, the number of known oncogenic viruses is increasing rapidly, and it is not unlikely that one or more forms of human cancer may subsequently prove to be induced by a virus. In the diseases where the development of lesions and symptoms is spread over a long period, the chance of arresting of suppressing the effects of the virus would appear to be greater than with an abrupt course of symptoms.

The points of the viral replication cycle which call for particular attention are those of enzyme synthesis and synthesis of the nucleic acid and structural proteins. If one considers the uninfected host cell, it is clear that only a small proportion of genes—encoded as sequences of nucleic acid—are actually active at any one time. There must be, therefore, a mechanism which selectively switches genes on or off; a proposal as to how this is done has been put forward by JACOB and MONOD (MONOD et al., 1963). If this can be achieved within the host cell nucleic acid sequences, then it seems possible that some differentiation may be achieved eventually between viral nucleic acid and host cell nucleic acid.

In some experimental virus-induced animal neoplasms at least, the maintenance of the malignant state apparently is not dependent on continued replication of the virus. Nevertheless, there is evidence that part of the viral genome is present and is presumably active. The problem here is the choice of an agent blocking expression of certain viral genes rather than one preventing replication.

In testing for antineoplastic agents, it is to be expected that the use of cells transformed by oncogenic viruses will increase. Apart from these, however, other cell-virus systems may also give useful information (HUEBNER et al., 1962) (TRENTIN et al., 1962).

The activity of a drug may be due to an active metabolite or complex formed in vivo, and therefore liable to be missed with in vitro studies. DICE and colleagues (1965) avoided this difficulty by combining in vivo with in vitro testing; they gave the drug to animals and subsequently took blood samples for assay in vitro. Rats were used rather than mice to obtain adequate volumes of serum. The doses level chosen was LD_{10} since the agent should be selective and not too toxic; in the initial stages four dose levels were given intraperitoneally. If activity was observed the substance was then tested by other routes of administration. As their primary screen, these authors chose herpes simplex, parainfluenza-3, measles and poliovirus using standard methods of assay.

In the testing of large numbers of substances for possible antiviral activity, metabolic inhibition tests are both convenient and rapid though less accurate than plaque reduction tests.

An agar-diffusion technique has been used for in vitro assay with plaque-forming or focus-forming viruses (RADA et al., 1960; HERMANN et al., 1960; SIMINOFF, 1961). After applying an overlay of agar to the infected monolayer, test agents are applied to the surface on discs or in cups. After incubation for a suitable time, the cultures are fixed and stained; plaques are then counted to determine whether there has been any significant inhibition of growth. It is possible also to apply paper chromatography to an agar surface to demonstrate differences in activity by various fractions.

This type of test is simple, rapid and inexpensive, and in the same specimen toxicity directed against the cell can be measured whilst studying the antiviral effect (LINK et al., 1965). By adding neutral red dye to the culture, cell damage is seen as a narrow zone of unstained cells centred around the point of application of the drug, whilst the wide zone of stained plaque-free cells indicates the antiviral effect. Link et al. (1965) used this method for testing against vaccinia, Newcastle Disease, Western Equine encephalitis and Rous sarcoma viruses. This systems also has the advantage that it can be used to demonstrate synergism between two antiviral agents; this is done with two paper strips soaked in the two substances and placed on the agar

overlay at right angles. Alternatively, it can be used to show reversal of an antiviral effect by some other agent. From bacteriology, KUCERA and HERRMANN (1966) have successfully adapted the gradient plate technique.

OXFORD and SCHILD (1967) have used organ cultures for the assessment of antiviral agents for rubella virus; this method may allow closer approach to in-vivo conditions than the conventional monolayer techniques.

With in-vivo trials, quantitation of effect is a matter of some difficulty. LINK et al. (1965) used influenza virus (Al strain) instilled intranasally in mice (in groups of 10 to 30) and observed daily for ten days. The cumulative percentage mortality was shown and plotted into a log probability net against the number of days after infection. JOHNSON (1965) suggest that in-vivo trials should include study on the effect of a drug on contact spread of a disease such as influenza in mice, thus stimulating natural conditions. The subject of development of drug resistance by viruses has been reviewed by SCHNITZER (1966).

Anticancer Screens

Antitumour testing has presented even greater difficulties. The wide screen used by SKIPPER and his colleagues (SKIPPER and SCHMIDT, 1962) is designed to cover as many different types of tumour and aspects of tumour metabolism as possible. Nevertheless the range of clinical variations seen with even a single type of neoplasm raises doubts as to how many test tumours one should use to stand a reasonable chance of picking up a majority of active substances from a pool. It seems inevitable that some antitumour agents, active perhaps against a limited range of neoplasms, must escape both in vitro and in vivo nets.

In 1953, the American Cancer Society sponsored a project for screening for possible anticancer drugs; this screen included animal tumours and viruses, bacteriophages, fungi, slime moulds, mammalian and avian embryonic cells, and Drosophila. No single tumour type is known to be capable of acting as a single-system screen (GELLHORN and HIRSCHBERG, 1955), neither can a non-tumour system act as the only screen for carcinostatic drugs. Although transplantable neoplasms may differ markedly in their metabolic patterns and responses from their original parent tumour, yet the modified cells may possibly share some particular biochemical feature with a spontaneous cancer of different origin. Whilst spontaneous tumours of even one histological type may differ in behaviour between themselves, and even individual growths change their response (e. g. to hormones) in course of development, yet there may well be many points of overlap in the metabolic patterns presented by various tumours.

HIRSCHBERG (1963) emphasised the difficulty of deciding on a sufficiently comprehensive range of criteria for testing after reviewing reports on the responses of 479 experimental tumour systems to various compounds. SCHEPARTZ et al. (1967) are now using sarcoma 180, adenocarcinoma 755, leukaemia 1210 and KB cells in culture together with some tests carried out on Friend virus leukaemia, Lewis lung carcinoma, human sarcoma H.S.1, Walker 256 (intramuscular), hepatoma 129, Cloudman melanoma (S.91), Murphy-Sturm lymphosarcoma, Dunning leukaemia (ascites) and P-1798 lymphosarcoma.

EAGLE and FOLEY (1958) reported a positive correlation between cytotoxicity in vitro and antitumour activity in vitro. This conclusion was based on a study of

200 substances tested against a number of cell lines, and was supported by the results of SCHEPARTZ et al. (1961) after investigation of a much greater number of compounds. SKIPPER (1964) has discussed the choice of criteria in the design of techniques for measurement of the effect of antineoplastic drugs.

The induction of tumours by viruses offers a convenient means of obtaining malignant growths for use in testing. In the event of viruses being identified as a cause of some human neoplasms, then transformation of human cells in vitro would appear to be of great potential value; that such transformation in vitro can take place in human cells has been demonstrated.

PIENTA, BERNSTEIN and GROUPÉ (1963) have ingeniously combined antiviral and antitumor testing, by checking in the first place for activity against the virus-induced Rous sarcoma tumour in vivo. This short-circuits a great deal of in vitro work; indeed the activity of xerosin can only be detected by an in-vitro test. Similarly, CHIRIGOS (1964) studied the use of leukaemogenic viruses in mice. GLYNN et al. (1963) used Moloney leukaemia virus and showed that leukaemic cells and the virus were differentially sensitive to the drugs used; they concluded that unless both virus and cells were destroyed by a compound, then erradication of leukaemia was impossible. Testing against both viral and tumour systems, JOHNSON (1965) points out that whilst vincristine and vinblastine are both active against neoplasms, the former is effective in vivo against Mengo virus but the latter is completely ineffective.

The disc plate method introduced by MIYAMURA (1965) allowed very rapid assessment—within eight hours—using the degree of inhibition of methylene blue reduction by Ehrlich ascites cells in an agar medium as an index of cytotoxic activity. The value of this approach is shown by the later work of YAMAZAKI et al. (1965) and DI PAOLO and MOORE (1957). The method has been adapted by MIYAMURA and NIWAYAMA (1959) for HeLa cells in agar medium, and again by SCHUURMANS et al. (1960) for S180 cells who allowed for growth of cells during the assay.

In the technique described by SIMINOFF and HURSKY (1960) and GRADY et al. (1960), the cells are grown on glass and the monolayer then overlaid with agar. The test substance is placed on the agar on discs and the extent of toxicity shown by fixation and staining. However, it is necessary to remove the agar after fixation and any dead or injured cells, and ROSENOER (1966) points out that this may lead to difficulty in reading the results.

The cell culture tube dilation broth assay method of EAGLE and FOLEY (1956) has been modified by SMITH et al. (1959 a). A standard amount of a cell suspension of known density is added to culture tubes, with or without the agent under test. After incubation, the final cell density—in terms of protein concentration—is compared in test and control cultures, the activity of the drug being assessed by the degree of inhibition of cell protein synthesis. A minimal difference of sixfold between the test and control is recommended as the dividing line for a potentially useful agent. FOLEY and EPSTEIN (1964) have made a comprehensive survey of the use of cell cultures in screening for antitumour agents.

BHUYAN et al. (1962) used three methods in comparing the activities of various compounds and found no direct relationship between the results obtained by different techniques. The methods were those of RENIS et al. (1962), which is dependent on the removal of damaged cells from a collagen plate, that of EAGLE and FOLEY (1956), and that of MIYAMURA (1956). A further development is that of GOLD (1966) who has

measured the differential anaerobic glycolytic rates of elements in a solid piece of tissue without disturbing the architectural mass. The technique is suggested for testing the differential effect of drugs on both the malignant and corresponding normal tissues, and might be extended to procedures other than anaerobic glycolysis.

An alternative method is that of SCHUURMANS et al. (1964) who use Sarcoma 180 and Detroit 6 cells suspended in an agar layer. Paper strips bearing potential antineoplastic agents are placed on the agar for a time, and the the cell preparations are incubated for two days. The effect is assessed by measurement of cellular dehydrogenase activity.

Three types of neoplasms can be used in vivo—spontaneous and induced (generally transplantable) animal tumours, and human cancer cells grown in conditioned animal hosts. The question of methods of drug evaluation has been comprehensively reviewed by ROSENOER (1966) who has discussed the use of various types of tumour for tests in vivo. ROSENOER points out that it is unwise to rely on published data for the growth characteristics of the tumour selected; a careful preliminary study is essential, using the same conditions which will operate during testing. The Therapeutic Index is LD_{50}/MCD; MCD is the Mean Carcinostatic Dose.

Assessment of the effect of a carcinostatic drug in vivo frequently depends on the rate of survival of the animals, but a more sensitive measure is change in weight. Both tumour inhibition and host toxicity tests can be combined in the same experimental animals (ROSENOER, 1966). VOGEL (1961 a, b) compares the degree of inhibition of bone marrow with the degree of inhibition of tumour growth. BROSS and TARNOWSKY (1962) used a "Toxicity Differential Index" based on the differential rates of increase of tumour inhibition and host toxicity. Using this Toxicity Differential Index, MOUNTAIN et al. (1966) tested 14 drugs against 8 rodent tumours; they considered the method had wide applications but pointed out certain limitations.

Another approach is that of SKIPPER et al. (1963) who introduced the idea of a "Specificity Index"; this depends on the difference in weight between control and treated tumour-bearing animals.

HANDLER et al. (1964) studied the reaction to antineoplastic drugs of transplantable tumours which metastasised in a regular manner; sometimes the primary tumour was inhibited yet the secondaries were not suppressed; such a system could be of value in testing agents for use in advanced cases. Similarly, KARRER et al. (1967) suggested the use of the Lewis lung tumour implanted into mice, since metastases occurred regularly whilst primary tumours were still small.

The general position will be improved as variations in metabolism are defined between types of tumour, and between tumour and normal cells. The example of mouse leukaemia cells dependent on an exogenous supply of L-asparagine from neighbouring normal cells spotlights the advantages which could be derived from such knowledge by stimulating a search for a substance with specific chemical properties. In this instance, the activity was found first (in serum) and the precise identification of the substance responsible followed later.

The Cancer Chemotherapy National Service Centre (1964) has introduced a programme for testing antineoplastic drugs for possible use clinically.

The mechanisms underlying drug resistance of tumours has been reviewed by BROCKMAN (1963), HUTCHISON (1963), VENDITTI and GOLDIN (1964) and ELION and HITCHINGS (1965). VENDITTI and GOLDIN (1964) point out that by combining drugs

the onset of resistance can be delayed. However, WEBB (1963) emphasised that when two inhibitory agents act on the same metabolic pathway, no greater therapeutic effect is produced by the two than by one only; RUBIN et al. (1964) confirm this.

The subject of development of drug resistance by viruses has been reviewed by SCHNITZER (1966).

The study of methods suitable for assessment of the response of individual tumours to antineoplastic drugs deserves more attention. DICKSON (1966) has introduced a filter-well technique which has the advantage that the interdependence between epithelial and stromal cells is not destroyed in vitro. A simple organ culture technique has also been devised by YARNELL et al. (1964) for study of the effect of various agents on human tumours.

Although antineoplastic drugs should seemingly be tested against neoplastic cells, yet the use of microbial screening systems have proved to be of value. The simplicity of a screen based on the reaction of micro-organisms has considerable appeal. SCHABEL and PITILLO (1961) have reviewed their use.

Foley et al. (1958) obtained most encouraging results; they found that using as few as four selected systems for the testing of 89 compounds 95% of these substances with antineoplastic activity in vivo also inhibited microbial growth. Some two thirds gave false positives which is not of great importance; only 5% gave false negatives.

GAUSE et al. (1959) has developed the use of biochemical mutants of bacteria as a screen based on the similarity to certain metabolic features of oxidation found in tumour cells.

For the assessment of hormones likely to be of value in the treatment of cancer, BECKER et al. (1963) worked with Physarum polycephalum. LEIN et al. (1962) used the ability to induce lysogenic bacteria as a method of detecting potential anticancer agents, and a similar approach was used by ENDO et al. (1963) who tested many antibiotics, antimetabolites and other substances. More recently GELDERMAN et al. (1966) examined the response of lysogenic bacteria to antineoplastic drugs and reported that in each case the combination of drugs suggested by the bacteriological test was more effective in its antitumour action than the use of one drug alone.

3. Microbial Sources

Substances active against both neoplasms and viruses have been isolated from many species of microorganisms. NEUSS et al. (1957) consider that about 0.5% of all cultures of microorganisms screened against solid tumours show some reproducible activity. In spite of this, however, out of many hundreds of cultures showing some degree of antineoplastic effect, comparatively few have reached any advanced stages of testing.

Actinobolin

The site of action of this antibiotic, derived from a Streptomyces culture and known to be active against experimental leukaemias, has been studied by SMITHERS (1966). Its primary action is inhibition of protein synthesis, and suppression of DNA synthesis follows as a secondary effect.

Actinogan and Peptinogan

Actinogan is a high molecular weight substance isolated from a species of Streptomyces (SCHMITZ et al., 1962), which shows activity against some experimental rodent tumours (BRADNER and SUGIURA, 1962). Peptinogan (of molecular weight 15,000) is evidently the active moiety of actinogan (SCHMITZ et al., 1963); it has a better therapeutic index, improved stability and greater solubility.

Actinomycins

The actinomycins form a group of related substances, the first being isolated by WAKSMAN and WOODRUFF (1940) from a species of Streptomyces. Aurantin is a mixture of a number of actinomycins and its properties have been described by PLANELLES et al. (1964).

Actinomycins contain an aminoquinone group giving rise to free radicals which attack the sulphydryl groups of protein and possibly other targets (KNOCK, 1967). The essential biological action of actinomycin is its combination with DNA (REICH, 1963). HASELKORN (1964) found, however, that there is no binding to either polyribonucleotides or molecular hybrids of DNA and RNA. In vitro, the formation of RNA on a DNA-template through RNA polymerase is inhibited (GOLDBERG and RABINOWITZ, 1962; HURWITZ et al., 1962; FRANKLIN, 1963; GOLDBERG, REICH and RABINOWITZ, 1963); however, at the same concentration of actinomycin the activity of RNA-dependent RNA-polymerase is unaffected (HURWITZ et al., 1962). It seems that the main effect of these drugs results from linkage with either guanine-cytosine pairs or of a sequence of guanine-cytosine and adenine-thymine (GELLERT et al., 1965).

GOLDBERG and REICH (1964) have suggested that actinomycin inhibits the RNA polymerase by virtue of positioning in the minor groove of the DNA polymer. Thus RNA formation is prevented by the complexing of actinomycin with DNA-templates (BURCHENAL and KREIS, 1967).

Interference with the synthesis of protein is also recognised. Since mammalian messenger RNA is fairly stable, GARREN et al. (1964) consider that inhibition of the formation of enzyme protein in mammalian cells is probably due, at least on some occasions, to blockage of the translation of RNA rather than with its synthesis. However, whilst both RNA and protein synthesis may be inhibited, the formation of RNA is suppressed at concentrations of actinomycin which give no effect on the production of antibody protein. Interferon formation is inhibited (GIFFORD and HELLER, 1963; WAGNER, 1964; HO and KONO, 1965), as is also the synthesis of histone in Sarcoma-37 cells (HONIG and RABINOWITZ, 1964).

A useful method for assessing the activity of an actinomycin is based on inhibition of the growth of HeLa cells (REICH et al., 1962). In sensitive lines of HeLa cells in vitro, a specific inhibitory effect can be achieved with concentrations as low as 0.001 μg/ml (JOURNEY and GOLDSTEIN, 1961).

HACKMANN (1952) was the first to report anticancer activity by an actinomycin (D). A number of these agents have been shown to be effective against several experimental animal tumours, differences in effect resulting from the use of different actinomycins. In general, the response of leukaemias and solid tumours is less satisfactory than that of ascites tumours (BURCHENAL et al., 1960). A table summarising the positive results is given by STOCK (1966).

Clinically, actinomycin is of established value. Indeed, on a molar basis, the actinomycins are the most active anticancer drugs available (KNOCK, 1967). It has frequently been used effectively in cases of Wilms' tumour in children (TAN et al., 1959; FARBER, 1960; FERNBACH and MARTYN, 1966) and regression of malignant lymphoma has also been reported. HOSLEY et al. (1962) found it to be useful, when combined with irradiation, in lung cancer, and REEMTSMA et al. (1959) used it in the treatment of breast cancer by regional perfusion. Beneficial effects have also been reported in the treatment of metastatic choriocarcinoma (ROSS et al., 1962), and testicular tumours (LI et al., 1960), in the latter when combined with methotrexate and chlorambucil.

MACKENZIE (1966) studied the use of actinomycin-D in the treatment of 154 patients with metastatic cancer from primaries in the testis. He considered it to be the most effective chemotherapeutic agent when used alone for dealing with secondaries from embryonal carcinoma, teratocarcinoma and choriocarcinoma, but inferior to chlorambucil for metastic seminoma.

Actinomycin has also been tried for effect against osteosarcoma, malignant melanoma, gastric and intestinal carcinoma (KNOCK, 1967).

A combination of actinomycin therapy with irradiation has proved to be of value in the treatment of rhabdomyosarcoma, neuroblastoma and sarcoma botryoides (FARBER, 1959; TAN et al., 1960). Also, actinomycin D has been used to potentiate the effects of radiation therapy in the treatment of Wilms' tumour in children (KNOCK, 1967).

BROCKMAN (1963) reported that tumours do not easily develop resistance to this drug. Actinomycin D has been used by KEIDAN (1966) in the treatment of 31 children with different types of malignancy. Nineteen had Wilms' tumour; many of these also received radiotherapy so that assessment of the effect of the drug was difficult. In the other 12 patients, some showed transient improvement. Toxic effects were frequently observed. This drug may potentiate the action of X-rays but may at the same time increase the risk of radiation nephritis and pneumonitis.

It is given intravenously and tissue necrosis results if it escapes into extravascular tissues. Toxic effects on marrow, liver and kidneys may appear some days after the end of the course and nausea, vomiting, anorexia, stomatitis and diarrhoea may also occur. The dosage is either 15 μg per kilo body weight for 5 days (which may be repeated in 2 to 4 weeks, or 10 μg per kilo for seven injections). Whilst nausea and vomiting can be controlled by chlorpromazine, other toxic effects determine the cessation of treatment.

STOCK (1966) has discussed the variation of toxicity with chemical structure. Actinomycin can suppress an immune response experimentally, but the effect depends not only on the dose but also on the timing in relation to the administration of antigen.

SCHAFFER and GORDON (1966) have studied the inhibition of growth of poliovirus by this agent and find that the degree of inhibition differs according to the strain of the virus.

When tested against influenza virus (PONS, 1967), it is found to be effective if given within the first $1^1/_2$ to $2^1/_2$ hours after infection; it is thought that it may effect the synthesis of viral RNA.

The effects of actinomycin D on the synthesis of RNA by avian myeloblastosis virus and BA1 strain A have been reported by ZISCHKA et al. (1966), who found it active against the virus only in doses which were toxic to the host cells.

Alanosine

This agent has been isolated from a Streptomyces culture (Str. alanosinicus nov. sp.), and shown to have both antiviral and antitumour properties (MURTHY et al., 1966).

A marked antitumour effect was demonstrated using a transplantable fibrosarcoma in hamsters induced by SV-40 virus, although no in vitro action was observed against the virus itself.

In vitro, activity was recorded against enteroviruses, vaccinia, cowpox and sheeppox, and in vivo significant protection was demonstrated in rabbits given neurovaccinia even when treatment was started as late as 24 hours postinfection.

GALE and SCHMIDT (1968) have investigated its mode of action. The synthesis of RNA is disturbed, possibly through the conversion of inosine monophosphate to adenosine monophosphate. Alanosine has been identified as L(—)2-amino-3-nitroso hydroxylamino-propionic acid.

Alpha Sarcin

This polypeptide (MW 16,000), derived from Aspergillus giganteus, is of interest since it contains a hitherto unknown aminoacid "sarcinine" (of undetermined structure) which is also present in two other antitumour peptides obtained from Aspergillus and is associated with the antineoplastic activity. It is effective in inhibiting the growth of several types of mammalian cells in vitro, and of a number of animal tumours in vivo (OLSON and GOERNER, 1965; OLSON et al., 1965).

Anisomycin

Protein synthesis is reversibly inhibited in HeLa cells by this substance (GROLLMAN, 1967) isolated from cultures of Streptomyces. Its chemical structure has been established and it has been shown that its inhibiting action occurs following the formation of aminoacyl transfer ribonucleic acid, but before the release of polypeptides from the polyribosome.

Anthramycin

Anthramycin is the active constituent of Refuin, and is a derivative of a thermophilic actinomycete (TENDLER and KORMAN, 1963; LEIMGRUBER et al., 1965 a, b).

Following trials against mouse tumours in vivo, and human neoplastic cells in vitro, KORMAN and TENDLER (1965) tested it clinically; they reported at that stage some cases of irreversible shock.

KORMAN (1967) considers anthramycin to be less toxic than a number of other neoplastic drugs, and recorded a positive response in about two-thirds of a group of 86 patients with advanced cancer. It may be of some use in breast carcinoma, but seems unsuitable in lung cancer and malignant melanoma. It is given by slow intravenous infusion in doses up to 1 mg per day.