Microbiology and Cancer Therapy: A Review*

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The use of micro-organisms and their products as possible therapeutic agents in the control of cancer had its beginning in the latter part of the nineteenth century, coinciding with the embryonic achievements of the science of bacteriology. The rapid advances in the field of antibiotics during the past 12 years have inspired new hope that the search among biological systems will disclose a chemical agent which will exert a destructive effect upon neoplastic growth without seriously affecting normal cells.

BACTERIA

The early history of the role of bacteria and bacterial toxins in this development has already been well reviewed (42, 101, 119, 145). Many of the investigations were prompted by the clinical observation that a concurrent bacterial infection frequently retarded the development of malignant processes in man. Some were based on the belief that organisms isolated from neoplastic tissues were the causative agents of cancer (39). Attempts were made to prepare vaccines and serums against such organisms; the efficacy of these preparations is highly doubtful. Although beneficial results were sometimes claimed, attempts to confirm such observations were usually unsuccessful; certainly no cures were effected.

The first record of an attempt to treat human tumors with an induced bacterial infection is that of Busch in 1868 (19). He had observed temporary clinical improvement in two patients with inoperable sarcomas who also developed concurrent erysipelas infections. His attempts to induce the infection in other cancer patients were unsuccessful, because the causative agent of erysipelas was not known at that time. Later, Fehliesen (38), after he had discovered the streptococcal origin of erysipelas, injected live cultures of the bacterium into cancer patients with encouraging results. Since then many varieties of micro-organisms have been reported to be beneficial therapeutic agents. Preparations known as "Coley's Toxins" (100, 101), which have usually consisted of heat-treated mixtures of streptococci and Serratia marcescens, have been used clinically for many years, although their effectiveness has not been clearly established.

Beebe and Tracy (13) reported that injections of suspensions of Bacillus prodigiosus (S. marcescens) cells, either alone or combined with streptococci, resulted in rapid and complete disappearance of transplanted lymphosarcoma in three dogs. Baroni (8) obtained some measure of protection against the growth of the Jensen tumor in rats if the animals had received injections of streptococcal or gonococcal toxins for several days prior to the implantation of the tumor. Simpson and Marsh (127) were unable to demonstrate any effect, either macroscopic or microscopic, upon the growth of spontaneous mammary adenocarcinoma in mice with tuberculin preparations.

Daels (27) and Comsia (23) found that spirochete infections in mice interfered with the development of tumors. The effect was most pronounced when the animals were infected with the spirochete several days before the tumors were implanted.

Connell (24) reported clinical improvement in several cases of far advanced cancer upon treatment with sterile filtrates of Clostridium histolyticum. The filtrates were prepared by cultivation of the bacterium on pieces of human malignant tissue.

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suspended in normal saline and were thought, by virtue of the method of preparation, to contain proteolytic enzymes which would preferentially attack the proteins of neoplastic tissue without affecting those of normal tissue. Although Connell mentioned that a carcinoma in mice responded to similar treatment, other investigators (29, 53, 106, 114) in well controlled studies with several animal tumors have been unable to confirm his observations. Procedures such as that employed by Connell have proved to be invaluable for the microbial production and isolation of enzymes possessing specific activities (34). They are based on the idea that when a microbial culture is supplied with a particular organic substance, either as the sole source of carbon or nitrogen or both, the organism, in order to grow, must attack that substance. The digestive process may require the formation of an unusual enzyme system or the production of an excessive amount of an ordinary system. It is highly doubtful, however, that such a method can prove satisfactory when an organism is supplied with such a heterogeneous mass of material as is present in whole tissue of either malignant or normal growth. It would seem probable, therefore, that only if and when a substance which is not present in normal cells is found in malignant tissue and is obtained in a purified state that this technic may offer real promise.

Parker et al. (104) injected Cl. histolyticum spores directly into a rapidly growing transplanted fibrosarcoma in mice and, with the administration of histolyticus antitoxin, prolonged the life span of some animals for as long as 20 days beyond that of nontreated tumor-bearing mice. Histolyticus toxin injected directly into the tumor caused marked regression of both a sarcoma and a carcinoma; in tissue culture the toxin produced severe damage to sarcoma but not to either carcinoma or normal kidney.

Cohen et al. (22) incubated slices of a spontaneous mouse carcinoma, the Brown-Pearce rabbit carcinoma, and their autologous livers in cultures of various bacteria. In general, the bacteria were found to injure both the liver and tumor cells equally well or not at all. Only one organism, Sporosarcina ureae, damaged the tumor cells but not the liver cells. The active factor appeared to be intracellular; the cell-free culture medium in which the bacterium had been grown was ineffective.

In vitro technics such as that employed by Cohen have somewhat dubious value in the search for agents which might be useful in the control of cancer. Such methods are frequently defended on the basis that when tissues are tested for their response in isolated systems, possible interfering effects of the whole animal body on the compounds being studied are eliminated. Although this is undoubtedly valuable in certain studies of particular biological systems, the application of any agent to cancer therapy depends to a great extent upon its fate in the living animal. Materials which are active in vitro may exert no demonstrable effect in vivo (71, 135). Conversely, an agent may cause marked response in vivo but be inactive in vitro (117). It is not inconceivable that enzyme systems in the body may alter compounds in such a way as to either increase or decrease their biological activities. Some agents may have very low therapeutic indices, and, although they exert pronounced effects upon tumor growth in vitro, the dose levels necessary to bring about responses in vivo can never be attained.

Gregory, who believes all cancers to be virus-induced (49, 50), has claimed to have seen by electron microscopy in vitro destruction of the “cancer virus” caused by Bacillus subtilis Tracy 1 (51). By chemical and electrophoretic technics, he obtained from extracts of the bacterium a crystalline material to which he has given the name magnesium tracinate. The crystals were administered to eight patients with far advanced cancer; all were reported to show clinical improvement. The possible merits of tracinate must await more extensive clinical trial, but it would appear to be somewhat presumptuous to attribute any effect that it might have upon neoplastic growth to antiviral properties until more decisive evidence as to the nature of the causative agent or agents of cancer is available.

In 1951 Gratia and Linz (47) reported that Bacterium coli filtrates injected into guinea pigs with sarcoma elicited the Shwartzman reaction (125) in the tumor tissue with no observable hemorrhagic lesions in other organs. Motivated by this observation, Shear and his associates began a study of the nature of the hemorrhage-producing agent (122). Shear and Andervont (129) concentrated the factor from B. coli filtrates by a method used for the precipitation of the soluble specific polysaccharide from pneumococcus broth cultures; the active fraction was found to give negative biuret and positive Molisch reactions (120).

The group of investigators at the National Cancer Institute later turned their attention to studies on the hemorrhage-producing properties of Serratia marcescens. Shear and Turner (124) obtained active preparations which were rich in polysaccharides. Further experimentation confirmed the polysaccharide nature of the material (56) and indicated that its molecular weight is about 8 million (69). Preparations of the purified polysac-
charide were found to produce hemorrhage in mouse tumors in doses of a fraction of a microgram (121). More recently, Ikawa et al. (86) have reported the isolation of a hemorrhage-producing agent from filtrates of E. coli which they characterized as a complex polysaccharide containing both a peptide and a phospholipid component. Upon treatment of this material with trichloroacetic acid, most of the peptide component could be removed; the lipo-polysaccharide which remained was found to contain most of the original tumor-destroying activity. Thus, it would appear that the peptide portion is not essential for activity.

Brues and Shear (17) administered the polysaccharide from S. marcescens to four patients with advanced inoperable malignant tumors. In two of the cases, some relief from symptoms was obtained, and, on post mortem examination, evidence of hemorrhage in the tumors was found. Gross changes in tumor size and consistency as well as microscopic evidence of necrosis and hemorrhage in both animal (30, 31) and human tumors (68, 108, 112) have been reported.

The usefulness of bacterial polysaccharide as a chemotherapeutic agent would appear doubtful. No human tumor has been completely destroyed and rather severe symptoms of toxicity have been encountered (68, 108). Nevertheless, it is to be hoped that studies with the polysaccharide may contribute to the knowledge of the treatment of neoplastic diseases.

Gardner et al. (40) produced hemorrhagic reactions in a transplantable rat tumor with extracts of a variety of bacteria including the Neisseriae group, Klebsiella pneumoniae, and enteric organisms. Jacobs (67) has reported similar effects on mouse Sarcoma 37 with a polysaccharide-containing fraction from Pseudomonas aeruginosa.

Although the role of bacteria in cancer therapy has intrigued the minds of clinician and research investigator alike for several decades, no real insight into the mechanics of the interference of tumor development by bacterial products was available until the observations of Gratia and Linz (47) on the Shwartzman phenomenon in guinea pig tumors aroused new interest. Shwartzman and Michailovsky (126) and Duran-Reynals (35) have reported the isolation of a hemorrhage-producing streptococci, have had therapeutic effects upon tumors, it must be concluded that the mode of action of such organisms would be quite different from that of the gram-negative bacteria.

**FUNGI**

Fungi, too, have received a fair share of attention as organisms possessing therapeutic properties against cancer. A brief discussion of some of the early attempts in this field has been given by Fichera (39). Karo (70) claimed to have obtained both subjective and objective improvement in several cases of human cancer as the result of treatment with fermentation products of the mushrooms Agarius rufus, Echinacea, Merulius laeryms, and Phallus impudicus which had been detoxified by extensive ultraviolet irradiation and to which several metallic salts were added. No details of the procedure of preparation were given nor was any mention made of what role the metallic salts themselves might play beyond the statement that they increased the therapeutic effectiveness of the fungal proteins.

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retard markedly the growth of Sarcoma 180 in
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It is equally distressing that the procedure
documents employed in the preparation of the fungal
extracts were not described.
Hulshoff Pol (84) has reported the regression of
tumors in mice on a diet containing bread upon
which the fungus Penicillium spinulosum Thom
had been grown.
DeAngelis (28) has claimed very good results
against Ehrlich’s adenocarcinoma implanted in
mice after treatment with a crude culture filtrate of
Streptothrix felis D.A. which he isolated from a
granuloma in a cat. The material, which he named
“mycetin,” was passed through a bacteriological
filter and administered in doses of 0.2–0.4 cc.; the
MLD was 0.5 cc. Injections were usually made
subcutaneously but at some distance away from
the site of the tumor; most animals received only a
single dose. Of a total of 330 treated mice bearing
tumors ranging in age from 12 to 25 days, tumors
regressed in 308 or about 93 per cent of the cases.
All 286 control mice died as a result of tumor devel
opment.
In 1946 Stock et al. (135) initiated a program for
the systematic screening of materials of natural
origin for their ability to inhibit the development
of tumors. Crude culture filtrates of several fungi
were found to have a destructive effect upon Sar
coma 180 and mouse melanoma tissue in vitro and
to cause inhibition of the growth of Sarcoma 180
and of mammary adenocarcinoma E 0771 in vivo.
Reilly and Stock (110) isolated from the metabolic
filtrate and pellicles of some strains of Aspergillus
fumigatus a protein-like agent having the ability to
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mice. The tumor-arresting property was accom
panied by a generalized toxicity; efforts to sepa
rate the two factors were unsuccessful. Petermann
et al. (105), in electrophoretic studies, located the
active principle in a group of highly basic protein
components.
That certain yeasts do have some effect upon
neoplastic growth seems probable. Laclau and
Imaz (75) reported that hydrolysates of Sac
charomyces cerevisiae which were biuret-negative
had an inhibitory effect upon neoplasms in human
beings. Laclau et al. (76) reported good results in
healing epitheliomas by intramuscular administra
tion of hydrolysates of Saccharomyces cerevisiae
combined with selenium preparations. The role of
the yeast preparation here is open to some quest
ion; the authors themselves attribute the effects
observed to the formation of dissociation products
of selenium.
Nevorojkin (102) found that when a dense sus
pension of the yeast Saccharomyces cerevisiae No.
XII (Berlin race) in normal saline was injected
directly into the Ehrlich tumor in mice, the growth
of the tumor was delayed, and, in some cases, re
gression occurred. Thin suspensions of the yeast,
however, not only failed to depress growth but,
apparently, even stimulated it. Maisin et al. (87)
studied the effect of heat-treated bakers’ yeast
upon the development of benzpyrene-induced tu
mors in mice. The yeast, suspended in water, was
heated at 80°C. for 6–7 minutes; the mixture was
added to a control diet so that each mouse re
ceived 0.25 gm. of yeast per day. At the termina
tion of the experiment in the group of 100 control
mice there were 51 survivors with an incidence of
tumors of 48.1 per cent, of which 23.2 per cent
were malignant. At the same time, in the yeast-
treated group (100 mice), 60 were still living.
These had an incidence of all tumors of 28.3 per
cent and an incidence of cancer of only 1.2 per
cent.
In in vitro experiments Protti (108, 109) ob
served that tumor tissues immersed in liquid cul
tures of a large variety of yeasts and incubated at
30°C. for 48 hours or at 37°C. for a shorter period
of time showed marked lysis; normal tissues were
unaffected. A selective action was noted; certain
varieties of yeasts affected some of the tumors but
not others.
Lewisohn and his associates (88) reported that
an aqueous extract of brewers’ yeast caused regres
sion of spontaneous mammary adenocarcinoma in
mice. Of 33 animals treated, tumors disappeared in
eight and were reduced in size in another ten; in
fifteen mice either there was no change or the tu
mor increased in size. Further investigation (86)
disclosed that the reported active principle was
water-soluble, fairly stable to heat at neutral pH,
and was not a protein. None of the known B vita
mins, including thiamine, riboflavin, pyridoxine,
nicotinic acid, pantothenic acid, and p-amino-
benzoic acid, appeared to be responsible for the
antitumor property (77, 84), although the addition
of either pantothenic acid or riboflavin to an active
yeast extract appeared to improve the effective-
ness of the latter in prevention of the development
carcinoma 2163 in the RIII strain of mice. Later,
inositol (78) and “L. casei factor” or folic acid
(79, 80) were reported to be relatively potent
inhibitors of tumor growth. Sugiura (138), however,
was unable to confirm the inhibition of sponta-
aneous mammary adenocarcinoma by yeast ex-
tracts or folic acid, although he made a special
effort to follow the procedure of Leuchtenberger
and associates. Indeed it is difficult to understand
the highly favorable results reported with folic
acid, since it would appear that certain of the folic
acid antagonists have a pronounced deleterious
effect upon neoplasms (134).

Laslo and Leuchtenberger (77) found that
some lots of brewers’ yeast did not contain the
antitumor factor. The deficiency was attributed to
a change in the conditions under which the yeast
was grown. While this explanation seems reason-
able, the earlier observations of Heaton (60) on
this problem cannot be disregarded. This investi-
gator found that extracts of brewers’ yeast con-
tained an agent which inhibited both normal and
tumor growth. His observation that different
samples of the yeast varied in activity, coupled
with the fact that extracts of bakers’ yeast did not
cause the effect, led him to think that the active
principle was not a product of the yeast but was a
constituent of the medium on which the organism
had been grown. Further experimentation indi-
cated that malt, a constituent used in the cultiva-
tion of brewers’ yeast, was actually the source of
the growth-arresting agent; results similar to those
seen with the active yeast extracts were obtained
with commercial malt extract.

Protozoa

Roskin and Klyueva (72, 113) found that infec-
tion with the protozoan Trypanosoma cruzi caused
regression of tumors in mice. As a result of more
than 15 years of investigation they claimed to have
separated from cells of T. cruzi an agent having the
ability to destroy malignant neoplasms in man as
well as experimental tumors in mice. Although
Malisoff (88) reported confirmation of these find-
ings in his work with two mouse tumors, a sponta-
aneous mammary carcinoma and transplanted
Sarcoma 180, a number of other investigators (57)
have been unable to prepare active T. cruzi lysates.
Hauschka et al. (59), in excellent studies, were able
to retard significantly the growth of three trans-
planted mouse tumors by active infection of the
animals with T. cruzi, but preparations of endo-
toxin (heat-killed cells) were without effect. These
results were open to some question, because the
work had been done with a strain of T. cruzi which
was different from that used by the Russian work-
ers. Hauschka and Goodwin (58) later extended
the investigations, including in their studies five
types of mouse tumors, both implanted and sponta-
aneous, and eight different strains of T. cruzi,
including the strain used by Malisoff and the one
which was, according to Roskin and Klyueva,
the source of the K-R factor. Again, they were unable
to obtain a tumor-destroying preparation.

In 1948 Gruhzit and Fiskin (52) reported fail-
ure to obtain any effect upon the development of
the Brown-Pearce carcinoma in the rabbit with a
lysate of T. cruzi. Jedeloo et al. (68) were unable to
demonstrate any inhibition of the development
and subsequent growth of a tar-induced epider-
moid carcinoma in mice with endotoxin prepara-
tions of T. cruzi. Spain et al. (132) obtained nega-
tive results with whole culture lysates of T. cruzi in
the treatment of mammary carcinoma and trans-
planted Carcinoma 119 in mice.

Nadel and Greenberg (99) have reported an in-
crease in survival time in leukemic mice when
Plasmodium bergei, the infectious agent of malaria,
was inoculated into animals after leukemia had
developed.

Antibiotics

For the purpose of this discussion antibiotics
will be considered as defined by Waksman (144):
“An antibiotic is a chemical substance, produced
by microorganisms, which has the capacity to in-
hbit the growth and even destroy bacteria and
other microorganisms, in dilute solutions.”

Although Boyland (16) found that some aro-
matic sulfur compounds which have antibacterial
properties retarded the growth of spontaneous
mammary tumors in mice, there is no a priori rea-
son to expect antimicrobial agents to be effective
against tumors. Nevertheless, the highly specific
nature of the action of antibiotics (143), that is,
the ability of such substances to inhibit the growth
of one micro-organism without affecting another
closely related one, even within the same species,
indicates the possibility that some of them might
display a similar selection between normal and
neoplastic cells. Certainly, there is evidence that,
under special conditions, such may be the case.

Over 40 years ago, Uhlenhuth et al. (141) and
Beck (12) reported that pyocyanase, an antibac-
terial agent now generally considered as the first
antibiotic to be isolated, caused regressions when
injected directly into tumors implanted in mice and rats. Unfortunately, as pyocyanase is a rather toxic agent, this line of investigation did not progress very far.

With the advent of penicillin, an essentially nontoxic chemical of remarkable therapeutic benefit in the control of certain infectious diseases, it was only natural that the attention of those interested in the control of cancer should be turned in this direction. Cornman (25, 26) observed that in tissue culture crude penicillin preparations exerted a lethal action upon cells of mouse and rat tumors at concentrations that did not damage normal cells. Although she confirmed Cornman’s result with crude material, Lewis (82) found that highly purified penicillin had no effect upon tumor growth in tissue culture. Other investigators (48, 91, 135) have reported similar experiences.

Beard (11) has reported that the administration of crude penicillin to rats bearing implanted Emge sarcoma apparently reduced the number of takes and caused regression of a high percentage of tumors. In three experiments with a total of 82 rats treated with penicillin, disappearance of the tumor occurred in 35–72 per cent of the animals, while regressions in 148 untreated control rats was less than 20 per cent. Dobrovolskaia-Zavadskaiia (32) has obtained histological evidence that yellow sodium penicillin does interfere with the growth of tumors in mice; the outstanding characteristic was the development of a massive hyperemia in the neoplastic tissue. The effect was temporary; when treatment was discontinued, the tumor reestablished itself. Those areas which had been subjected to treatment, however, appeared to be permanently damaged. In one patient with mammary adenocarcinoma, she (33) saw marked clinical improvement under penicillin therapy and observed histological changes similar to those seen in mice. Bennison (14), on the other hand, could find no difference in the incidence of the development of mammary tumors in mice carrying the milk factor with treatment of impure penicillin. Stock (133) has reported that even at a dose of 5 gm/kg body weight/day crystalline penicillin G had no effect upon the growth of Sarcoma 180 in vivo.

It is of interest that Burk et al. (18), in studies of effects on the metabolism of tissues, observed that several preparations of amorphous penicillin, varying in potency from 1,000 to 1,500 Oxford units/mg, brought about a marked inhibition in the respiration of several tumor and normal mouse tissues, while crystalline penicillin G (1,660 Oxford units/mg) was only one-tenth as active on a weight basis. Burk considered it possible that the slight activity shown by the highly purified material might be the result of the presence of a small amount of impurity.

Levine et al. (81) isolated from culture filtrates of *Penicillium notatum* a nonpenicillin-containing material which they called penichromin. In Warburg studies with mouse liver homogenates penichromin inhibited the oxidative utilization of several intermediary metabolites but did not affect succinoxidase, cytochrome oxidase, or anaerobic glycolysis. *In vivo*, the preparation had no effect upon the growth of an adenocarcinoma or a lipoma in mice. These investigators concluded that “in view of the *in vitro* data showing partial inhibition, the failure of penichromin alone to produce tumor regression was not unexpected.” This conclusion would not appear to be fully justifiable on the basis of the evidence presented. Treatment in mice was not begun until about 1 week after implantation of the tumors, and only one dose level, 2 mg/mouse daily for 5 days, was used. No data were presented to indicate the relationship of this dose to the maximum tolerated dose. It is possible that the utilization of younger tumors and higher doses of penichromin might have given more promising results.

In view of the few encouraging reports on the effects of crude penicillin on tumors and observations in our own laboratories of quite definite but elusive inhibitory effects of crude culture filtrates of *P. notatum*, it seems probable that a more thorough and systematic investigation of this problem might prove fruitful.

Notatin, a second antibacterial agent produced by *Penicillium notatum*, was chosen by Carr (20) as being of possible interest in the control of the Rous sarcoma virus. He reasoned that it might be effective, because the action of notatin has been shown to be the result of the production of H2O2 in the oxidation of glucose to gluconic acid (115) and because, according to Carr’s interpretation of the data of Gye and Purdy (54), the Rous virus is readily destroyed by oxidation. This second reason would appear to be erroneous because, as Gye and Purdy pointed out, their experimental evidence does not warrant a decision as to whether the loss of potency of the Rous virus was the result of oxidation or was brought about by destructive proteolytic enzymes. Regardless of the possible error in his hypothesis, Carr did find that, *in vitro*, complete loss of virus activity could be accomplished when the virus was mixed with a solution containing notatin and glucose. When, however, notatin was injected into fowls either before inoculation with virus or after development of tumors, no effect upon either the incidence of infection or growth of the tumors was observed. Chinn (21) re-
ported similar in vitro effects upon the Rous virus with terramycin, aureomycin, neomycin, and antibiotic PA96 (Pfizer). When, however, chickens, previously infected with virus, were treated with aureomycin or terramycin, the course of the disease was not altered. In fact, with low concentrations of the antibiotics there appeared to be a stimulatory effect upon tumor development.

Stock et al. (138) found that immersion of the tissue in a solution of clavacin (1 mg/cc) completely destroyed the viability of Sarcoma 180 and a mouse melanoma, while streptomycin (10 mg/cc) appeared to have no effect. Gliotoxin, in an aqueous suspension of 1 mg/cc, caused an 80 per cent reduction in the growth of treated Sarcoma 180 implants but had no effect upon the melanoma.

Stock (138) later reported that clavacin and gliotoxin had only very slight, if any, effect upon the development of Sarcoma 180 in vivo.

Kidd (71) found that cells of two mouse tumors and of the Brown-Pearce rabbit carcinoma were rendered nonviable by immersion for a short time in crude culture filtrates of the fungus Aspergillus fumigatus. As a result of comparison experiments, he indicated that the active principle in his fungal filtrates was possibly identical with gliotoxin. He was unable to demonstrate any effect with potent culture filtrates upon the development of the Gardner lymphosarcoma in mice. Mason and Kidd (90) concluded that the cyclic disulfide linkage which is present in gliotoxin is necessary for the destruction of tumor cells in vitro.

Vollmar (142) observed that malignant cells in tissue culture were inhibited by patulin (clavacin) at concentrations that not only did not damage normal tissue but actually stimulated its growth, a factor which might be of interest in wound healing.

Barnard and his associates (6, 7) reported that, when the usual course of therapy yielded no benefit to two patients with leukemia, the oral administration of a crude fermentation concentrate, which was obtained from Chas. Pfizer & Co., resulted in both clinical and hematological response. This was at first attributed to the high concentration of vitamin B12 in the concentrate, but further investigation concluded Barnard to conclude that not B12 but rather any of the streptomycetes-derived antibiotics (terramycin, aureomycin, streptomycin, or chloramphenicol) could bring about temporary remission in leukemia (6, 7). Goldman (46) observed no alteration in the clinical course of the disease in five patients with Hodgkin’s disease upon the administration of aureomycin. Ayres (4) treated thirteen cases of what he termed “anaplastic lesions (canceroma in situ) of the cervix” with topical applications of aureomycin. He claimed to have obtained complete regressions in six of these patients.

The diagnoses of these cases are open to severe question, and the malignant nature of the lesions is highly doubtful. It is most probable that any lesions which may have responded to aureomycin therapy were infectious, not cancerous, in nature. Further, it is significant that, in five cases of advanced carcinoma, Ayres noted no real beneficial effect upon the administration of aureomycin.

Bateman et al. (10) employed aureomycin as an adjuvant in the treatment of cancer patients who were receiving x-ray and massive HN2 therapy. Administration of the antibiotic was followed by gross and nonspecific microscopic effects on the tumors and in some cases appeared to potentiate the effects of x-ray and HN2. Five cases which had previously been considered as inoperable were modified sufficiently to permit surgical treatment.

Malmgren and Law (89) found that aureomycin, chloramphenicol, and streptomycin did not affect the tumor-producing properties of the mammary tumor milk agent in C3H mice. When the milk agent was injected into strain C mice, the data suggested that streptomycin may have activated the virus; the mean tumor age in the antibiotic-treated group was somewhat lower than that in the control animals. Ambrus et al. (2) have found that aureomycin, terramycin, and chloramphenicol have no inhibitory effects upon the development of several tumor-inducing viruses either in vivo or in vitro.

Sokoloff and Eddy (128) observed a very definite stimulation of implanted carcinoma in rats fed a small amount of aureomycin (1.6 mg/day/100 gm body weight for about 1 week). Large doses (8 mg/day/100 gm), however, caused a marked inhibition of tumor growth as well as severe loss in total body weight.

Stock (138) reported that of eleven antibiotics tested only two, actinomycin and citrinin, caused reproducible slight inhibitory effects on the growth of Sarcoma 180 in mice. Reilly et al. (111), in a survey of 38 antibiotics, found that only five—actinomycin, actidione, illudin M, illudin S, and terramycin—caused a slight retardation of the development of Sarcoma 180 in vivo.

Hackmann (55) found that, when suspensions of the Ehrlich ascites carcinoma, mouse Sarcoma 37, or the Walker rat carcinoma were mixed and incubated for a short time with actinomycin C, the viability of the tumors was severely damaged. In vivo the antibiotic retarded slightly the growth of the Walker rat carcinoma at dose levels which caused little or no toxicity in the host. Schulte (116) has reported preliminary results on 150 patients treated with actinomycin C, either alone or...
in combination with x-ray therapy. Although little response was observed in cases of carcinoma, the effects noted in over 50 patients with lymphogranulomatosis were reported to be encouraging. Doses of 50–250µg. of antibiotic were administered daily over a period of several weeks without harmful effects to the patients. These gained weight and showed an improved blood picture. External tumors disappeared rapidly; mediastinal tumors, however, showed only slight regression. As Schulte has emphasized, conclusions on the merits of actinomycin C as a therapeutic agent against cancer cannot be made at this time. His report comprises results obtained over a period of only 1½ years; in some cases the dose of antibiotic was probably too small. Tests on more patients at higher dose levels as well as post-treatment observations over a longer period of time will be necessary before actinomycin C may be properly evaluated.

Bateman and Klopp (9) have claimed that the concurrent administration of actidione and aminopterin significantly prolonged life in leukemic mice. Their enthusiastic conclusion, however, is not supported by convincing data; the survival of the treated mice was only very slightly greater than that of the untreated animals.

It would appear that any favorable responses which have been observed in the treatment of human cancer with clinically available antibiotics have been the result, not of the ability of such agents to destroy tumor cells, but of their capacity to improve the general condition of the patients temporarily by the control of certain secondary microbial infections. Nevertheless, the suggestive results obtained in laboratory animals with some antibiotics indicate the necessity for a continued search for a cancer-controlling agent among substances of this type.

**VIRUSES**

Although a complete analysis of the intricacies of virus interference phenomena is beyond the scope of this presentation, the potentialities of viruses as useful agents in the control of cancer merit some attention. Turner and Mulliken (138) and Moore (92) have reviewed briefly the historical background of this field, and only some of the more recent contributions will be considered here. Turner and Mulliken (138) found that, when brain-adapted vaccinia virus was injected into mice with Sarcoma 180, the virus localized in the tumor tissue. In early studies, infected tumors appeared to grow more slowly and to regress more frequently than untreated tumors. Later, however, these investigators concluded that the virus did not influence the growth of Sarcoma 180 appreciably (139). Moore (92) reported similar results with the viruses of influenza A and herpes simplex. Turner et al. (140) obtained some increase in survival time in leukemic mice treated with vaccinia virus, but the slight effect was lost when repeated passage of the leukemia rendered it more virulent (139).

Moore (92) demonstrated definite destruction of Sarcoma 180 in mice which had been infected with the virus of Russian Far East encephalitis. The tumor contained a high concentration of virus and failed to grow when transplanted to mice immunized against the virus. Examination of histological sections showed massive cellular damage. Further investigation disclosed that five other transplantable mouse tumors responded to the Russian virus in a similar manner (94). Koprowski and Norton (73) confirmed Moore's observations with the Russian encephalitis virus and showed further that certain other neurotropic viruses possess oncolytic properties. Toolan and Moore (137) reported that Egypt virus 101 had a destructive effect upon a human epidermoid carcinoma grown in x-radiated rats. Southam et al. (129) found that infections with West Nile and Ilheus viruses temporarily inhibited leukemic leukocytosis and infiltration in mice but did not cause any significant increase in survival time. The ability of neurotropic viruses to attack tumors would appear to be specific in nature; each virus has its own "tumor spectrum" (73, 96), a term coined to indicate that all oncolytic viruses do not destroy all tumors but that each virus is effective against only a particular group of tumors which vary with the virus employed.

Unfortunately, the tumor-necrotizing effects of the neurotropic viruses in mice have been achieved at dose levels of the viruses which cause eventual death of the animals. If active infection does not take place, the tumor is not affected (73, 98). Ordinarily, susceptible tumors when grown in mice immunized against the virus do not respond to virus therapy (98). Kuwata (74) has reported similar findings with the ornithosis virus and a mouse carcinoma. Such results present a very dim prospect for the practical application of viruses to cancer therapy. Nevertheless, the observations of several investigators offer some hope that the tumor-destroying properties of certain viruses need not necessarily be accompanied by destruction of nervous tissue. Sharpless et al. (118) found that the viruses of Russian and West Nile encephalitis could cause regression of a malignant lymphoid tumor in chickens without killing the host. Ginder and Friedewald (44) have reported rapid necrosis
of rabbit fibroma and some effect upon the development of myxoma in rabbits (45) by the Semliki Forest virus without causing death or apparent illness in the animals. Moore (97) has obtained destruction of Sarcoma 180 by the Russian encephalitis virus without killing the host when the tumor was implanted in PRI strain mice.

Moore (95) has attempted modification of the Russian encephalitis virus to increase its oncolytic properties and simultaneously to lower its affinity for nervous tissue. By repeated tumor to tumor passage of the virus she was able to obtain a strain of virus which destroyed Sarcoma 180 cells more rapidly than did the original strain, but its neurotropic properties remained unchanged.

Kuwata (74) has reported that Rickettsia tsutsugamushi and the ornithosis virus multiplied and persisted in two mouse tumors for several days. Although the infections appeared to have no effect upon the development of the original tumors, on bioassay heavily infected tumors showed loss of viability.

In spite of the apparently overwhelming obstacles that are involved in the use of viruses in the treatment of cancer (181), some cautious clinical studies have been made. Bierman et al. (15) reported some response in leukemia upon treatment with the virus of feline agranulocytosis. Southam and Moore (130, 131) have made very careful studies of the effects of several neurotropic viruses on the course of human neoplastic diseases. No permanent beneficial effects were achieved, but in a group of 34 patients treated with Egypt virus some temporary objective regression appeared in four, and suggestive but not conclusive beneficial effects were noted in five others. Higgins and Pack (61, 62) inoculated rabies vaccine into 30 patients with malignant melanomas. In at least six of the cases dermal metastases decreased in size and became flattened. The development of new metastases appeared to be retarded.

Although the practical application of viruses in cancer therapy thus far has yielded no outstanding results, experimental evidence indicates that the success of such treatment is not beyond the realm of possibility. A virus that showed a propensity for tumor tissue but was nonpathogenic or only mildly virulent for human beings might achieve results in the clinic similar to those which have been observed with chickens and rabbits. Also, if an antibiotic that was effective against an oncolytic virus were available, it might be possible to infect individuals with the particular virus, and, after a lapse of time sufficient to insure destruction of tumor tissue, to control any ensuing systemic viral infection with the antibiotic. The potentialities of the therapeutic use of viruses have not yet been exhausted and certainly deserve further consideration.

The mechanism of the action of the inhibition of tumor growth by viruses is not known, and, in view of the lack of factual knowledge on this subject, any attempt to explain this phenomenon can only be speculative. Clinical evidence indicates that it is not merely the result of nonspecific factors such as fever or stress (181). Although there is no experimental evidence to support such a theory, several investigators (94, 131, 138) postulate that the oncolytic effects of viruses may be brought about through competitive metabolic antagonisms. Any conclusions on this problem must await further data on the metabolism of both viruses and mammalian cells.

DISCUSSION

Micro-organisms offer an almost infinite source of new and interesting chemical compounds: many have the ability to form substances the very existence of which is beyond the wildest fantasies of man's imagination. In many instances even when the structure of a new microbial product can be elucidated, all efforts to manufacture it synthetically meet with defeat. Thus, it is not unreasonable that the search for an agent which will destroy neoplastic growth should be made among products of microbial metabolism. Such investigations, however, should be undertaken only when the complexities of the problem have been carefully considered and appreciated. The microbiological aspects alone appear quite formidable. As has been clearly pointed out in antibiotic research (143) and, to a lesser extent, even in cancer investigation (108, 109), the ability to produce a specific agent is a characteristic, not of a genus or species, but of a particular strain of micro-organism. In our own experience with seven different isolates of Aspergillus fumigatus, five were found to produce tumor-retarding filtrates, while two were completely inactive.

The now widely recognized variability of microbial cultures presents an added hazard. Some organisms, freshly isolated from natural sources, retain most of their original properties on repeated transfer on laboratory media; others very quickly lose their abilities to carry out the special functions for which they were isolated. To keep a culture in an active state, with respect to its capacity to produce a particular substance, requires great care and constant vigilance, which all too frequently is of no avail.

The nutritional and environmental conditions of cultivation markedly influence the metabolic
functions of an organism. Conditions that are optimum for maximum growth may not be suitable for the formation of a specific substance. Frequently an unknown precursor must be supplied, or a proper balance among certain mineral elements must be achieved. Oxygen tension, pH, temperature, and time of incubation must be carefully controlled. Although the number of variables seems most forbidding, the tremendous economic, industrial, and medical importance already achieved in the field of microbial fermentations (107) clearly demonstrates that they are not insurmountable obstacles.

In any attempt to find among natural sources agents that have specific properties either of an enzymatic, antimicrobial, or antitumor nature, one is confronted with the problem of whether highly purified preparations or crude mixtures, such as microbial culture filtrates, should be tested. Both offer definite advantages. Purified agents, even when their chemical structures are not known, are usually well characterized, and preparations from different batches, depending upon their degree of purity, do not vary greatly. Thus, any effects that they may exhibit can be more or less readily reproduced. On the other hand, most pure compounds that are biologically interesting have been isolated because they affect systems other than neoplasms, and, in the process of purification, any component that might possibly have been active against tumor growth has been lost. Such would appear to have occurred in the crystallization of penicillin.

Because they are heterogeneous mixtures, crude materials offer great possibilities. It is well recognized in the field of antibiotics that one microorganism may produce several antimicrobial agents simultaneously; this might well be true of the production of antitumor substances. The possibilities of additive or synergistic effects among several components in crude preparations certainly cannot be disregarded.

In any search for an agent that will be useful in the therapy of human cancer, a most disturbing element is the choice of test procedure and of test tumor. The tremendous strides in antibiotic research and development have been made possible to a great extent by the fact that preliminary tests for demonstrating antimicrobial activity and for following the production and chemical isolation of the active principle can be carried out in vitro. In cancer research, although in vitro technics do provide excellent tools for detailed studies of specific problems, their use as test procedures to pick up agents which must finally interfere with abnormal growth in vivo has not been very fruitful. Thus, it would appear that at present screening for antitumor agents must be conducted in vivo. This immediately excludes the possibility of the use of a really rapid and simple procedure.

In view of our scant knowledge, the choice of a test tumor must necessarily be arbitrary. Whether it should be a spontaneous or an implanted one has been a point of contention for many years. There can be no doubt that certain properties of transplanted tumors are different from those of spontaneous ones, even when the former have been derived from the latter (48). Nevertheless, transplanted tumors would appear to be the ones of choice for use in large scale testing programs because they are readily available and offer much greater opportunities for well controlled experimentation than do spontaneous developments.

The fact that different animal tumors have been shown to exhibit varied responses to treatment with chemical agents (41, 133) presents an additional problem. Some tumors may be markedly affected by a particular compound, whereas others will possess a strong natural resistance to it. Thus, any results that may be obtained with one type of tumor provide little or no information as to what may be expected with other tumors.

When all the factors that are involved in this field of investigation are considered, the need for patient and persevering endeavor becomes apparent.

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