transient increase in virus production by the tumor tissue itself when it is explanted *in vitro*. LASFARGUES and MOORE have availed themselves of this to follow in electron micrographs the evolution of mouse mammary carcinoma virus at the surface of the tumor cells[94].

In this short survey it is impossible even to touch upon all the many facets of cancer research as approached through tissue culture. References are therefore given to several reviews and general discussions in which the reader may find more meat and expanded literature lists[4,23,29,58,65,66,68,95–100]. Our aim has been to sketch in briefly some of the more important current trends and ideas.

If the panorama of tissue culture observations allows us to stress any single characteristic of the living cell, that would be its versatility. The same cell in culture can vary its metabolic pattern from respiration to glycolysis[32]; it can as a Schwann cell envelop an axis cylinder in myelination[101] or release itself and wander off like a macrophage[102]. It can as a myoblast manufacture the highly organized micellar bundles which characterize skeletal muscle[103,104], or it can perform the type of synthesis which produces the equally specialized mitotic spindle and new chromosomal DNA. These are not the same types of activity and apparently it cannot perform both at the same time. As a consequence, cells dividing rapidly over any considerable period tend to abandon, or suspend, their differentiating proclivities and lose many of their distinguishing marks. It becomes difficult to tell cells of normal lines from those of neoplastic lines in such cultures, where the biochemical equipment of both is preoccupied almost exclusively with the activities involved in multiplication—which do not seem to be tissue-specific. Genetic alterations attendant upon abnormal mitoses may confuse the picture further, and bring about permanent transformations from normal to malignant and vice versa, as well as many irrelevant changes.

Considering the potential fluidity of cellular behavior and the wide range of culture methods available, the future seems to hold hope for identifying and evaluating in the cell and in its environment some of the factors involved in steering the cellular generating mechanism from one type of activity to the other—from cytodifferentiation to proliferation, and back again.

Résumé

On donne ici un bref exposé des principaux courants d'idée qui se rattachent surtout à la recherche anticancereuse telle qu'elle a été abordée depuis la seconde guerre mondiale par la méthode des cultures de tissus. De grands progrès techniques ont aujourd'hui rendu possible la manipulation en masse de la cellule de mammifères en cultures continues, cellule traitée essentiellement comme microorganisme. Toutefois, comme les cellules à croissance rapide autant d'origine normale que maligne perdent leurs caractères individuels et finissent par se ressembler, l'intérêt se reporte actuellement sur les cultures de tissus organisés, où les facteurs favorables à la différenciation et au contrôle de la croissance peuvent être examinés.

Report on the Chemotherapy Section of the VIIth International Cancer Congress*

By W. DAVIS, London**

The VIIth International Cancer Congress held in London in July, 1958, showed how wide the developments of chemotherapy have now become in this field.

Clinical reports dealt with refinements in the mode of administration of existing drugs and the enhanced therapeutic results that ensued. The use of drugs as adjuvants to surgery earned attention too. At the same time, on the chemical side, many of the experimental papers dealt with newer drugs derived from already established parent compounds. This was especially true of the nitrogen mustards, but applied to several others in both the classes of alkylating agents and antimetabolites. A very few compounds were described that had not been derived from existing tumour-inhibiting drugs, but for none of these were there any substantial clinical claims.

The mode of administration of the earliest nitrogen mustard, HN2—di-2-chloroethylmethylamine—was by intravenous injection. This was the first clinical application of an alkylating drug and much of the later

[94] E. Y. LASFARGUES, D. H. MOORE, M. R. MURRAY, C. D. HAAGENSEN, and E. E. POLLARD, J. biophys. biochem. Cytol. *5*, 93 (1959).

[95] M. R. MURRAY, *Uses of Tissue Culture in the Study of Malignancy*, Ch. II, in *The Physiopathology of Cancer*, 2nd ed. (F. HOMBURGER, Ed., Paul Hoeber, New York 1959).

[96] M. R. MURRAY and A. P. STOUT, Texas Rep. Biol. Med. *12*, 898 (1954).

[97] M. R. MURRAY and A. P. STOUT, *Tissue Culture in Tumor Classification and Diagnosis*, in G. T. PACK and I. M. ARIEL, Eds., *Treatment of cancer and allied diseases* (Paul B. Hoeber, New York) *1*, 124 (1958).

[98] J. LEIGHTON, Cancer Res. *17*, 929 (1957).

[99] C. M. POMERAT, Ed., *Tissue Culture Technique in Pharmacology*, Ann. N.Y. Acad. Sci. *58*, 971 (1954).

[100] P. R. WHITE, Ed., *Proceedings of the Decennial Review Conference on tissue culture, Woodstock Vermont*, J. nat. Cancer Inst. *19*, 467 (1957).

[101] M. R. MURRAY, *Factors Bearing on Myelin-formation in vitro*, in *Progress in Neurobiology IV. Symposium on Myelin* (S. R. KOREY and J. I. NURNBERGER, Eds., Paul Hoeber 1959).

[102] P. WEISS and H. WANG, Proc. Soc. exp. Biol. Med. *58*, 273 (1945).

[103] H. HOLTZER, J. ABBOTT, and M. W. CAVANAUGH, Exp. Cell Res. *16*, 595 (1959).

[104] M. R. MURRAY, *Skeletal Muscle in Culture*, Ch. 5 in *Muscle*, Vol. *1* (Ed. G. BOURNE, Academic Press, 1959).

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development in this series was devoted to the production of related compounds that retained therapeutic activity, but shed the vesicant action of the parent drug. Many newer drugs could then be tabletted and administered orally. Both these modes of administration were of course going to produce a general concentration of the drug throughout the system, and this imposed an upper limit on dosages, since damage to bone marrow and the intestinal mucosae was bound to accompany any therapeutic effect. This in turn has limited the application of these chemotherapeutic agents to the systemic malignant diseases like the leukaemias and Hodgkin's disease. E. T. KREMENTZ (New Orleans) has now developed a method by which a chemotherapeutic agent may be administered locally by regional perfusion through an extracorporeal circuit. He is able to isolate a limb by closing artery and vein and perfusing with a bubble oxygenator. In experiments with dogs, he has developed techniques for perfusing the limb, the mid gut and the liver. Clinically the method has been applied in treating successfully a patient with lymphangitic recurrence of malignant melanoma in the leg. The doses of melphalan—p-di-2-chloroethylaminophenylalanine—thus administered would have produced marked systemic toxic effects had they been given generally.

In many papers and discussions, the role of chemotherapeutic agents in the management of neoplasms was considered in a general way. L. LARIONOV (Moscow) maintained that provided chemotherapy was started early enough and maintained as close as possible to maximum tolerated dose, it alone was capable of producing very long remissions in cases of leukaemia. L. HEILMEYER (Freiburg) had analysed survival time after diagnosis in patients with Hodgkin's disease and chronic myeloid and lymphatic leukaemia who received radiation, or chemotherapy or both. His results showed the longest survival time (54·5 months) for those patients receiving radiation alone. Combined radiation and chemotherapy gave an intermediate survival time (46·3 months), whilst for chemotherapy alone the time was reduced to 42·2 months. These figures tended to be heavily weighted in favour of radiation, since in that group were all the patients who responded satisfactorily from the start of that form of treatment.

The combination of chemotherapy with surgery revealed an important controversy. Theoretically, administration of a chemotherapeutic agent at the time of surgical excision of a tumour should serve to minimise the ever-present risk of metastasis. This had been demonstrated experimentally by H. SATO (Japan) who was able to suppress completely the formation of metastases in mice by administering nitromin following the surgical removal of tumours induced by subcutaneous transplantation of ascitic hepatomas. The clinical experience of B. RUBANYI (Budapest) who had used Degranol both pre- and post-operatively, and of R. GROSS (Marburg) who had used Endoxan post-operatively showed no undesirable side-effects nor any interference with wound healing. J. F. BINKLEY (Oklahoma, U.S.A.) who reported on ten years' experience of nitrogen mustard as an adjuvant to surgery was perhaps more equivocal in his estimation of the results. Moreover, in the discussion following these papers, cases were reported in which nitrogen mustard administration had interfered with normal granulation and others in which abscesses during healing had been encountered where excision of a bronchial carcinoma had been followed by chemotherapy.

Many of the chemical developments of the nitrogen mustards are attempts to achieve chemically what E. T. KREMENTZ is doing surgically, devices to take the active chloroethylamine specifically to its intended site of action. L. G. ISRAELS (Winnipeg) described clinical trials with just such a drug, CB. 1414 (4-di-2″-chloroethylamino-2-methylazobenzene-2′-carboxylic acid) designed by W. C. J. ROSS and G. P. WARWICK (London) to be active only after reductive fission of the azo-bond had occurred. Endoxan (N-di-2-chloroethyl 0, N′-trimethylene phosphorodiamidate) was synthesised by H. ARNOLD (Brackwede, Germany) with a similar rationale in mind. The intact molecule would be generally distributed after administration but, in those sites in the body where there was high phosphoroamidase activity, perhaps in the tumour itself, the active di-2-chloroethylamine would be released. Subsequent experiment has suggested, in fact, a different mechanism of action for this drug, but the rational approach of the alkylating agent with latent activity remains valid.

Another group of modified nitrogen mustards is exemplified by the phenylalanine nitrogen mustard, referred to earlier, the sarcolysine peptides and dopan. In these drugs the active chloroethyl moiety is attached in one case to an amino-acid, in another a peptide and the third a pyrimidine in the hope that the carrying structure might impart a certain selectivity of action, by bringing about some natural metabolic involvement of the drug. The sarcolysine peptides were reported by L. LARIONOV (Moscow) who claimed that they were able to produce a regression in certain transplantable tumours of rats and mice without causing depression of haemopoiesis.

Useful drugs have been produced by carrying the alkylating groups on a carbohydrate-like residue. A. ECKHARDT (Budapest) described the use of mannomustine (Degranol) in the treatment of malignant lymphomas and leukaemias. Degranol is 1:6-desoxy-bis-(2′chloroethylamino)-D-mannitol dihydrochloride and is much less toxic than other chloroethylamines. An analogous drug known as CB. 2511 was mentioned by A. HADDOW (London). This drug, made by G. M. TIMMIS and S. S. BROWN, is the 1:6-bis methanesulphonyl derivative of mannitol and is in a parallel

way much less toxic than busulphan (Myleran), which is bismethanesulphonoxybutane.

1:2-3:4 Di-epoxybutane had been recently re-examined by J. Bichel (Aarhus, Denmark), who demonstrated tumour inhibitory activity in both *meso*- and *dl*-forms. The *meso*-compound, however, was considerably more toxic and clinical trials, especially against Hodgkin's disease, were being carried out with the *dl*-form. I. S. Johnson (Indianapolis) reported tests on a number of basic bis-epoxides, for example, N,N'-bis (2:3 epoxypropyl)piperazine and N,N'dimethyl-N,N'-bis(2:3 epoxypropyl) tetramethylene diamine, which would prolong life in experimental mouse leukaemias. In clinical trials, I. H. Krakoff (New York) found some therapeutic effect with the piperazine derivative but the responses which did occur were all of brief duration. Many other clinical reports showed that TEM and Thio-TEPA, both alkylating drugs whose active grouping is ethyleneimino-, are still being widely used, but apart from the already well-established ethyleneimino-derivatives of benzoquinone (Bayer E 39 and A 139) no new drugs of this type were described. F. L. Rose (Macclesfield, Great Britain) had, however, attempted the preparation of the similar bis- or tris-diazomethyl-derivatives of 1:3: 5-triazine. These attempts were not successful, but 2-diazomethyl-4:6-diamino-1:3:5 triazine was made and did show inhibitory action against some experimental animal tumours.

Alongside the alkylating agents and receiving a similar amount of attention from both clinicians and experimental workers, the antimetabolites were widely reported. The clinical application of the most widely known drug in this field, 6-mercaptopurine (Purinethol) showed that it still commanded support, but some of the newer antimetabolites had aroused considerable interest. E. Frei (Bethesda, U.S.A.) had conducted clinical trials with 6-azauracil and he and his colleagues found haematological improvement in five out of sixteen patients suffering from acute leukaemia, but the high neurotoxicity of this uracil antagonist placed a serious limitation on its further application. With urethane, however, 6-azauracil displayed a marked synergism and as reported by Gertrude Elion (Tuckahoe, U.S.A.) it strongly potentiated the inhibitory action of urethane on the growth of the experimental tumour, Ca 755. The riboside of this same compound, 6-azauridine, has been synthesised by A. D. Welch (New Haven, U.S.A.) who described experiments in which the riboside was shown to have a wider spectrum of activity than the parent pyrimidine.

5-Fluorouracil and 5-fluoro-orotic acid had been synthesised and studied by R. Duschinsky, E. Pleven, and C. Heidelberger (U.S.A.). Both compounds exerted a profound antibacterial activity *in vitro* and inhibited a variety of transplanted animal tumours. Extensive biochemical studies had shown that 5-fluorouracil can actually become incorporated as its nucleotide to give a 'fraudulent' nucleic acid in various tissues, whilst at the same time tending to inhibit nucleic acid synthesis. Clinical evaluation of 5-fluorouracil was given by A. R. Curreri (Madison, U.S.A.). Of 15 patients with mammary carcinoma, 13 showed improvement after treatment as did 5 out of 18 cases of cancer of the colon and rectum. Some hepatomas and ovarian tumours also responded. The riboside and deoxyriboside of 5-fluorouracil have been synthesised and are under investigation.

There were unfortunately very few reports of searches for tumour inhibitory compounds beyond the main groups of alkylating agents and anti-metabolites. A. Furst (Stanford, U.S.A.) described the inhibition of the Ehrlich ascites tumour in mice with Kethoxal (α-keto-β-ethoxybutyraldehyde). He suggested that this compound with its adjacent carbonyl groups might antagonise chemically similar, known metabolites in the Krebs cycle. Other compounds of this type and their derivatives have been examined by F. A. French *et al.* (San Francisco, U.S.A.). They found that a number of dicarbonyl compounds including glyoxal, malonaldehyde, succinaldehyde and a number of α-ketoaldehydes were all active against experimental leukaemias and ascites tumours. Some of the hydrazones and oximes derived from the dicarbonyls also possessed carcinostatic activity, including glyoxal-bis-guanylhydrazone, glyoxal-bis-thiosemicarbazone, glyoxime and succinaldoxime. The most promising of these, glyoxal-bis-guanylhydrazone, is undergoing preliminary clinical studies.

Significant inhibition of mouse melanoma was obtained by V. Riley (New York), with *ortho*-, *meta*-, and *para*-phenylenediamine. He had already demonstrated a specific oxygen-absorbing reaction between these diamines and dihydroxyphenylalanine which is a melanoma metabolite. This reaction presumably also constituted a detoxication mechanism, for tumour-bearing mice, when challenged with these compounds, could tolerate far higher doses than could normal mice. Riley had, in fact, used this comparison to bring to light biochemical or metabolic distinctions between normal and tumour-bearing animals and considered that this approach offered diagnostic as well as chemotherapeutic possibilities.

The fundamental biochemical approach to tumour chemotherapy was made in the paper presented by Van R. Potter (Madison, U.S.A.) who discussed the application of radioactive intermediates to the study of differences in the metabolic pathways in normal and malignant tissue. S. Weinhouse (Philadelphia, U.S.A.) compared the respiratory properties of tumours especially in relation to pathways in glucose catabolism.

Abnormal enzymic activity in tumours was discussed for instance by G. Weber and A. Cantero

(Montreal) who noted the absence of fructose 1:6-diphosphatase activity in hepatomas, and by F. BERGEL (London) who argued the case of the involvement of enzymes and co-enzymes in carcino-chemotherapy in relation to xanthine oxidase and ribonuclease. The approach of M. B. SAHASRABUDHE (Bombay) fell in this same group. He argued that since adenine is a common precursor of both nucleic acid and the pyridine nucleotides, rapid synthesis of nucleic acid—such as exists in tumours—tends to be accompanied by a lowering of the pyridine nucleotide levels. Yet pyridine nucleotide is required for hydrogen transport and energy production, and so SAHASRABUDHE suggested that an alternative pathway involving hexose-monophosphate oxidation must be involved in tumour growth. Antimetabolites aimed at this pathway should then be specific antagonists of malignant growth. Such a compound, thiophene-2:5-dicarboxylic acid, had been shown to inhibit an experimental rat tumour.

J. SCHULTZ (Philadelphia, U.S.A.) found that experimental chloroma was extremely sensitive to Thio-TEPA—the triethylene thiophosphoramide. These tumours are extremely rich in porphyrin and it was found that tumours, otherwise refractory to this drug, could be sensitized by pretreatment with porphyrin. This approach offers wide scope for enhancing the effectiveness of already known anti-tumour drugs.

The overall picture of chemotherapy then, as it was presented at the Congress, was one of essentially slow step-wise development from already established knowledge, together with a small but important leavening of new ideas and approaches that would perhaps become the established positions of the future.

Zusammenfassung

Die Chemotherapie von bösartigen Gechwülsten und Leukämien wurde am VII. Internationalen Krebskongress in London sowohl vom Gesichtspunkt der Klinik wie von demjenigen der experimentellen Forschung aus behandelt.

Nahezu alle in diesem Zusammenhang erwähnten Pharmaka gehören entweder zur Gruppe der Alkylierungsmittel oder aber zu derjenigen der Stoffwechselantagonisten.

Das klinische Interesse galt vor allem der Verbesserung von therapeutischen Anwendungsweisen bereits anerkannter Heilmittel, deren Brauchbarkeit zur Unterstützung der Chirurgie, sowie der Prüfung neuartiger Substanzen mit möglicher Hemmungswirkung auf Tumoren.

Die Beiträge auf dem experimentellen Gebiet umfassten biochemische Studien über den Wirkungsmechanismus von Krebspharmaka und über den biologischen Einbau von Stoffwechselantagonisten. Einige Vorträge behandelten die Frage, welche Bedeutung den Enzymen in der Chemotherapie des Krebses zukomme.

Brèves communications - Kurze Mitteilungen
Brevi comunicazioni - Brief Reports

Konstitution des Cinobufagins[1]

Cinobufagin (I), das Hauptbufogenin der chinesischen Krötengiftdroge Ch'an Su, besitzt die Formel $C_{26}H_{34}O_6$. 5 der 6 Sauerstoffatome sind bisher in ihrer Funktion eindeutig abgeklärt worden und verteilen sich auf die folgenden Gruppen: ein sekundäres Hydroxyl (vermutlich an C–3), den Lactonring und eine Acetoxygruppe[2]. Letztere wird schon durch $KHCO_3$ verseift[3], woraus der Schluss gezogen wurde[4], dass diese an C–16 des Sterinskelets zu plazieren ist. Die Funktion des 6. und letzten Sauerstoffatoms des Cinobufagins konnte vor kurzem[4] auf spektroskopischem Wege geklärt werden: das IR.-Absorptionsspektrum des Acetylcinobufagins (II) wies eine gut sichtbare Bande bei ca. 3,31–3,32 μ auf, die der CH-Schwingung eines sekundär-tertiären Epoxyds zukommt[5–7]. Wir haben bereits die Vermutung ausgesprochen, dass diese Sauerstoffbrücke in Analogie zur Epoxy-Gruppe des Resibufogenins[7] an C–14/C–15 fixiert ist. Unter Berücksichtigung der oben angeführten Befunde käme somit für Cinobufagin die Formel I in Frage. Wie im Folgenden gezeigt wird, liess sich diese Vermutung exakt beweisen.

Bei der Oxydation von Acetylcinobufagin (II) mit $KMnO_4$ in Aceton war eine Säure $C_{24}H_{34}O_7$ erhalten worden, die als gut kristallisierter Methylester der Formel $C_{25}H_{36}O_7$ charakterisiert werden konnte[4]. Dieser wurde nun mit $LiAlH_4$ reduziert[7,8], wobei ein Tetrol gewonnen werden konnte, das nach Acetylierung eine kristallisierte Acetylverbindung gab, die auf Grund des Schmelzpunktes, der Mischprobe, der spez. Drehung und des IR.-Spektrums identisch war mit 3β,16β,20-Triacetoxy-14-hydroxy-21-nor-5β,14β-pregnan (III). III war durch analoge Reduktion des 3β,16β-Diacetoxy-14-hydroxy-5β,14β-ätiansäure-methylesters (V)[9] [aus Diacetylgitoxigenin

[1] Die ausführliche Publikation erscheint demnächst in den Helv. chim. Acta.

[2] Eine Literaturzusammenstellung der älteren Literatur ist vor kurzem gegeben worden[4].

[3] J. P. RUCKSTUHL und K. MEYER, Helv. chim. Acta *40*, 1270 (1957).

[4] J. P. RUCKSTUHL und K. MEYER, Helv. chim. Acta *41*, 2121 (1958).

[5] H. HENBEST, G. D. MEAKINS, D. NICHOLLS und K. TAYLOR, J. chem. Soc. *1957*, 1459.

[6] H. SCHRÖTER, CH. TAMM und T. REICHSTEIN, Helv. chim. Acta *41*, 720 (1958).

[7] H. LINDE und K. MEYER, Helv. chim. Acta *42*, 807 (1959).

[8] H. SCHRÖTER, R. REES und K. MEYER, Helv. chim. Acta *42* (im Druck).

[9] K. MEYER, Helv. chim. Acta *29*, 718 (1946).