ATTEMPTS AT CHEMOTHERAPY OF NEOPLASTIC AND RELATED DISEASES

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INTRODUCTION

As has been said often and will be pointed out on many future occasions, the sub-division of pharmaceutical agents into symptomatic and chemotherapeutic ones goes back to Paul Ehrlich and his time (*Table 1*). Since

Table 1. Pharmaceutical, iatro or therapeutic agents

Pharmacodynamic or symptomatic drugs	*Chemotherapeutic or aetiotropic drugs*	*Prosthetic or restitutional drugs*
e.g. CNS Depressants	*e.g.* Anthelmintics (some M.A.)	*e.g.* Metabolic products
CNS Stimulants	Antiprotozoal	incl. Proteins, amino-acids,
" Psychotropic drugs " (some M.A.)	Antibacterial (some M.A.) (Antibiotics)	nucleosides, minerals *etc.*
Drugs acting on autonomic effector cells	Antiviral (Interferon)	Holo- and coenzymes
Drugs acting on specific organs *etc.*	Anti-tumour (some M.A.)	Vitamins
	Anti-leukaemic *etc.* (some M.A.)	Hormones
		Immunological products *etc.*

M.A. = Metabolic antagonists

those days, with the first serious attempts at drug-design (how has this optimism changed to a more sober, I did not say, sombre mood) this sharp subdivision has been somewhat blurred by the fact that a third group of compounds has joined the other two, comprising those of a prosthetic or restitutional nature. Among the latter we find some compounds which though not usually classed as chemotherapeutic agents, might also be applied in chemotherapy proper, that is the removal, or at least temporary control, of the cause of a number of diseases by drugs.

BIOLOGICAL BASIS

This cause could be an invader as crude as a helminth or as subtle as a virus or it could be a changed host cell behaving as egocentrically as any foreign intruders, whether they may be bacterial, spirochaetal, protozoal or filtrable particles. Other meetings, and on this occasion other speakers, have discussed and will discuss the successful developments and the many victories of anti-microbial, anti-protozoal, and other anti-infectious drugs. Why is it then with all this experience behind them, that the scientists engaged in cancer research (and, one may add, in virology), have so much less pronounced practical successes to their credit and that their progress is relatively so slow? It is obvious that the main reason for this is that neoplastic and most virus diseases are bound up with internal events of cells and that the main problems (*Table 2*) facing the combined front of chemists–biologists

Table 2. Carcino-chemotherapy

by

Destruction	*Restitution* (?)
Problems of:	Problems of:
Transport to tissue and into cytoplasm and nucleus (vascularisation, permeability)	Essential cellular constituent
Selective concentration	How to resist fraudulent bio-synthetic stimulation
Selective toxicity	Availability of constituent
Anti -mitotic, -invasive (?), -metastic (?) drug actions	Transport and permeation

are the questions of transport to and into cell structures (closely connected with vascularization of the tissues and the permeability of cells to be attacked), and if killing-action or stasis is contemplated and hoped for, the question of selective concentration and toxicity. To these problems one has to add those of anti-growth or anti-mitotic effects on the so-called primary tumours and the most desirable, but as yet not realized, anti-invasion effects on the spread of malignant cells. If, on the other hand, it is possible in the future to find a therapy which might restitute missing essential cell constituents or which might help the cell to resist wrong biosynthetic stimulation exerted, *e.g.* by viruses or virus-like particles, then the discovery, availability and transport of such remedies (natural or imitatory in function) to and into the cells or nuclei may represent the main problems. You see, in the case of cancers the tissues and cells to be treated may not be wholly accessible to drugs or restitutional agents. This could be due to an increasing lack of vascularization of the internal parts of neoplasms, as demonstrated by Goldacre and Sylvèn[1], with the help of an easily penetrating dyestuff, lissamine green (*Figure 1*). The questions arise whether it is sufficient to rely on the attack of the drug solely on the outside of a tissue region or whether it is necessary, as

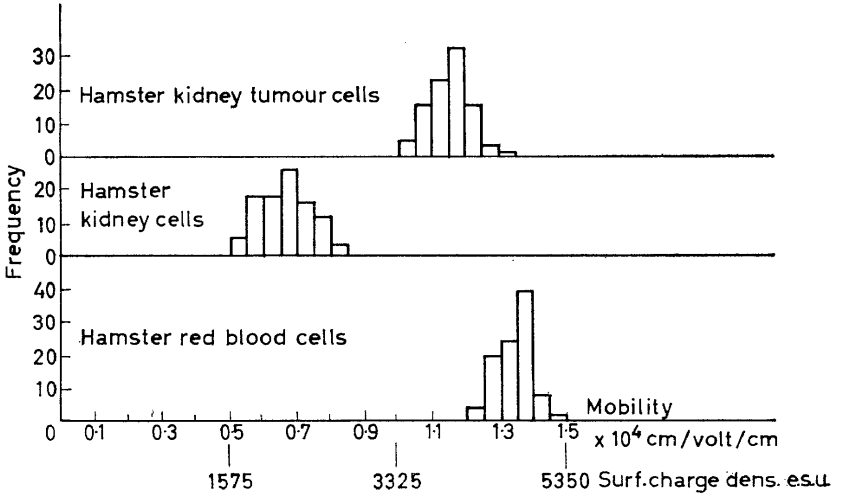


Figure 2. The surface charge density of normal and tumour cells
(By courtesy of E. J. Ambrose *et al. Nature* **177**, 576, 1956.)

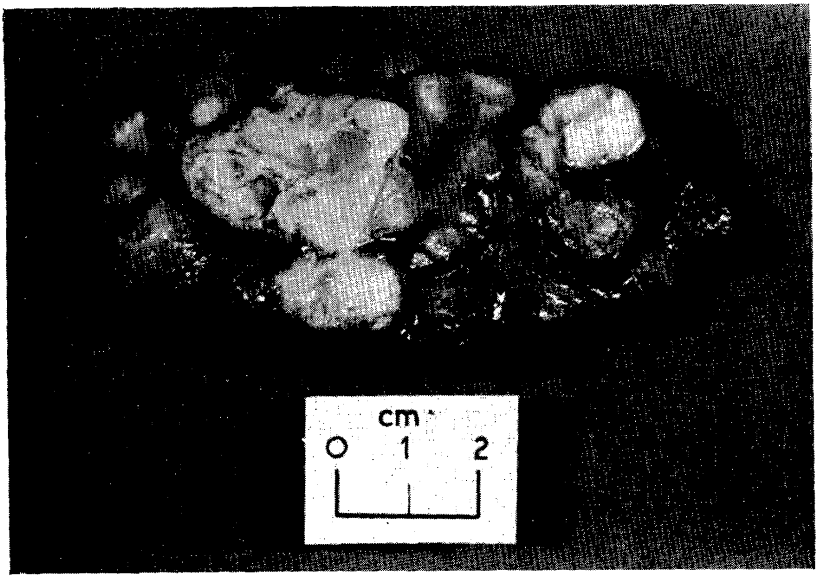


Figure 1. Walker tumour from rat treated with lissamine green (prepared at the Chester Beatty Research Institute)

recommended by a number of workers, to combine chemotherapy with surgery, *i.e.* removal of the main tumour followed by post-operative drug treatment; or whether intra-arterial infusions or regional perfusions would be procedures of choice. One could discuss for hours the problem of permeability of cells themselves, that is the passage of drugs across the cellular membranes of whole cells, of their nuclei (and sometimes mitochondria) by passive, facilitated or active transport, phagocytosis, as discussed recently by Quastel *et al.*[2], and pinocytosis; the latter, according to Holter[3], more often occurring with tumour cells than with normal ones. We could talk about events concerning the cell surface and its different charge densities (*Figure 2*) which according to Ambrose *et al.*[4] may have some bearing on spreading and metastasizing tendencies, but all this would take me away from the principal line of this paper.

BORDERLINE THERAPIES

However, before dealing with a few aspects of a quasi-rational approach to the preparation of chemicals, and of their properties and application to tumour-bearing animals and cancer patients, I must deal very briefly with matters which go, as some of you may think, beyond the confines of chemotherapy proper: they refer to (*a*) preventive medicine, (*b*) immunology, (*c*) virology and (*d*) endocrinology.

(*a*) It is not often remembered that those of us in cancer research who test organic and inorganic chemicals for their carcinogenic effects and try to relate chemical structure and physical properties of a series of compounds to their cancer-producing activity, study not only the possible origin of cancer and its development, but are concerned also with recommendations for removal of certain chemicals from manufacturing processes or from daily use either in foodstuffs, cosmetics or indirectly even in cigarettes or in the atmosphere (*Table 3*). In promulgating abstinence from or removal of

Table 3. Carcinogenic agents
for which controls have been proposed or for which regulations have been issued

Dye stuff intermediates, *e.g.* β-naphthylamine
Dye stuffs in food or cosmetics
Dulcin
Food preservatives, *e.g.* 3-amino-*s*-triazole (cranberries)
Cigarettes (smoke)
Petroleum and oil residues
Radioactive luminescent materials,
etc.

such sources and legislation for the control of specific compounds one is doing what could be called "negative chemotherapy" (to follow Boyland[5] when he was talking about hormone withdrawal). It should help in diminishing certain forms of cancer, such as bladder cancer due to β-naphthylamine and, maybe, lung cancer.

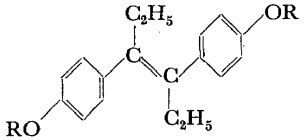
(*b*) When foreign macromolecules, mainly of protein or carbohydrate nature, and carried by invading organisms or viruses, are challenging certain host tissues they can induce the formation of antibodies. Cancer

cells derived from host cells might, in spite of this, antigenically speaking, do the same if they carried sufficiently different and determinant cellular constituents. Unfortunately, tissue or cells on carcinogenesis change, from an immunological point of view, in two directions which are somewhat opposing each other: they lose some normal and gain some new antigens[6]. It is the latter group which could represent a promise for a future immunochemical approach to cancer treatment. However, the antigenic potency of most of these tumour-specific constituents is low and their purification beset with great difficulties. However, this whole field on the fringe of cancer chemotherapy is on the move but there is no certainty as to its direction, and rapid and successful arrival. This was borne out by a recent discussion at Helsinki, under the auspices of the International Union against Cancer[7].

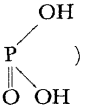
(*c*) Closely connected with all this are the activities of the virologists in cancer, with Stanley[8] and Gross[9] leading and with more and more workers probing into this fascinating problem. From the beginning it should be said that *up to now* there is no clear-cut evidence in favour of viruses being the causative agent of human neoplasms, except perhaps, and this is being investigated just now, in the case of malignant lymphomas prevalent among children in some regions of Africa[10]. (This should not throw any doubt on the virus aetiology of murine leukaemias, polyomas, rabbit papilloma, and fowl sarcomas). One has also to distinguish between viruses or virus-like sub-cellular particles as inducing agents, to be fought perhaps with anti-viral remedies, vaccines and anti-sera, and viruses as oncolytic agents. To touch on the latter first, Dr Alice Moore[11], among others, is studying this approach which she has reviewed from time to time. For the worker in chemotherapeutics it would be most important to learn something about that part of the anti-tumour virus which is "oncolytic". We may one day see the preparation or synthesis of polynucleotides, either of DNA, RNA or messenger RNA-character which could act like an oncolytic virus *solely* on tumour cells. On the other hand, if the development of a neoplastic state is directly or indirectly due to a virus, it might be possible to find, according to Harris, interferon-like materials[12] which could, at some future date, assist cancer chemotherapy. But at the present time this is more like a pipe dream after laboratory hours. In any case, virus-produced tumours, if ever discovered in man, ought to require, in most cases, *preventive* treatment, because after the full establishment of the tumour and achievement of independence from causative agents anti-viral treatment would have little chance of success except in recurrent infections.

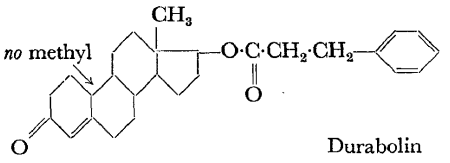
(*d*) The mechanistic rôle of hormones either in carcinogenesis or carcinotherapy is far from clear. Whether they influence enzyme action, protein synthesis or cell permeabilities, their application in the chemotherapy of leukaemias (cortisone, ACTH in acute forms), of breast and prostate cancer originated more in an empirical than in a rational manner. While in carcinoma of the prostate stilboestrol or stilboestrol diphosphate (*Table 4*) is still the drug of choice, breast tumours in women respond sometimes to oestrogens, sometimes to androgens and sometimes to adrenalectomy, hypophysectomy or pituitary-destroying procedures, all treatments called by Boyland[5], as mentioned before, "negative chemotherapy". The modern trend of steroid research aims at the preparation of steroid derivatives which

Table 4. Hormonal preparations



Stilboestrol

Stilboestrol diphosphate (R = )



Durabolin

possess only weak or no sex hormone activities but only antagonist action (see Durabolin).

CARCINO-CHEMOTHERAPEUTIC AGENTS

This brings us to more recent developments of anti-tumour and anti-leukaemic drugs proper to be found under these headings (*Table 5*): Biological alkylating agents, anti-metabolites and enzyme inhibitors, and a

Table 5. Groups of carcino-chemotherapeutic agents

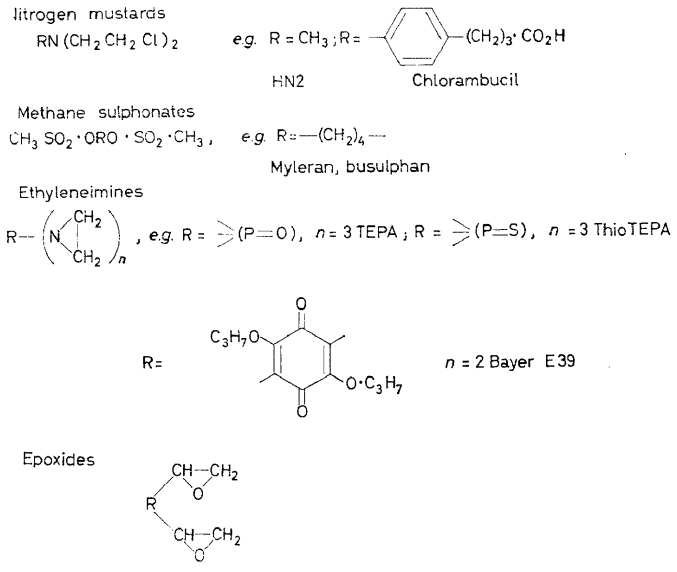
(1) Biological alkylating agents
(2) Antimetabolites and enzyme inhibitors
(3) Replacement attempts:
    purified enzymes
    enzymomimetics
    other cellular constituents
(4) Products from micro-organisms and plants:
    antibiotics, colchicine, vincaleukoblastine, *etc.*

group of potential remedies, in the true sense of the word, which in form of purified cellular constituents and enzyme models or enzymomimetics may help to change the aberrant cell into one which could be less dangerous in its behaviour to the organism as a whole.

Another group, (4), which I cannot discuss here, are natural products from micro-organisms or higher plants (including antibiotics, colchicine, and vinblastine).

(1) There is no necessity to bore you with the well-known history and details of the main types of biological alkylating agents (*Table 6*), the nitrogen mustards, methane sulphonates, ethyleneimines and epoxides. What I wish to present to you today will touch on two principal directions in the studies of this group which in its clinical application is, so one has to confess, of use but a limited one; these agents act by destroying growing cells without possessing true selective toxicity nor a pronounced effect on the spread of cancer by invasion and metastases. One of these studies aims at better knowledge of the mechanism of action of these drugs, the other at improvement inside the limits set by their over-all properties. Arising from researches carried out by two of my colleagues, Brookes and Lawley[13], it is probable that the mutation-, anti-tumour and carcinogenic effects of these

Table 6. Biological alkylating agents
(By courtesy of the *British Medical Journal* from F. Bergel, ii, 399, 1961.)



agents are due mainly to their alkylation of N^7 of the guanine moiety of DNA, (*Figure 3*) effecting a quaternization of this ring nitrogen and then a distortion or breakdown of the strands of the double helix; in the case of bi-functional alkylating agents these are supposed to react with guanine

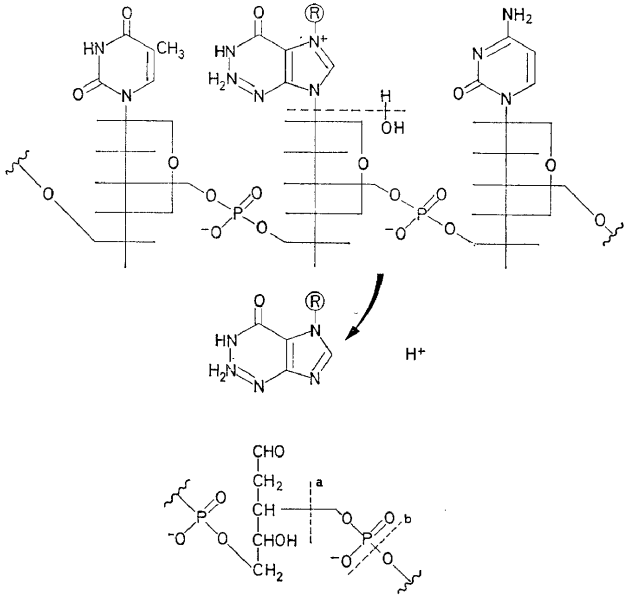


Figure 3. Alkylation mechanism of nucleic acids
(By courtesy of P. D. Lawley. *The Molecular Basis of Neoplasia*, p.126, University of Texas M. D. Anderson Hospital and Tumor Institute, 1962)

moieties of different strands (*Figure 4*). The other advance refers to the work of two other colleagues, Roberts and Warwick with "Myleran", a dimethanesulphonate, which in the rat seems to interact with thiol groups of

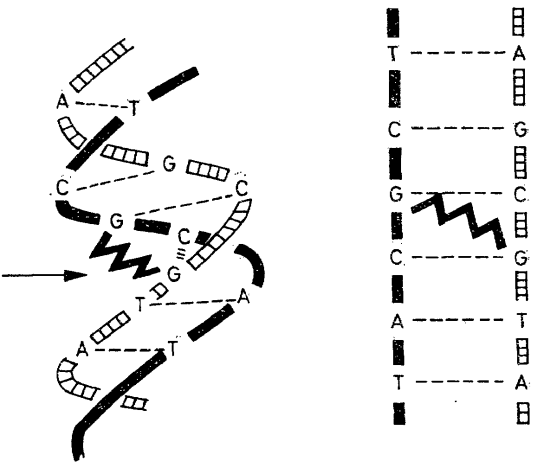


Figure 4. Alkylation mechanism of nucleic acids
(By courtesy of P. D. Lawley. *The Molecular Basis of Neoplasia*, p.127, University of Texas M. D. Anderson Hospital and Tumor Institute, 1962)

essential proteins and peptides such as reduced glutathione. These interaction products break down with loss of functional thiol compounds to give a hydroxylated sulphone of tetrahydrothiophene in the excreted urine (*Figure 5*). It is of course not absolutely certain yet whether these events are

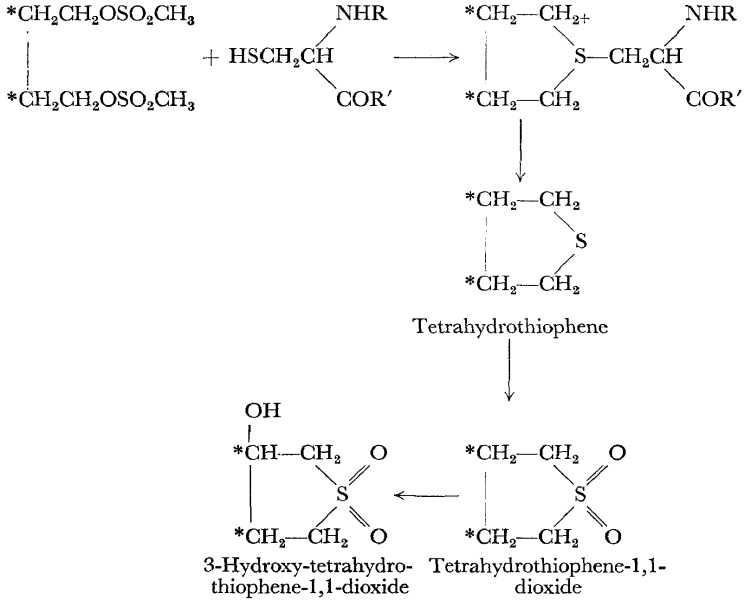


Figure 5. The metabolic conversion of Myleran *in vivo*
(By courtesy of J. J. Roberts and G. P. Warwick. *Ann. Rept. Brit. Cancer Campaign* **38**, 13, 1960)

causally connected with the biological effects of these agents but they ought to help in our understanding of some of the mechanistic details of action and in the continued search for improved drugs.

(2) Such search is vital if one wishes to keep pace with the increasing demand by the clinician for carcino-therapeutic agents which he is desirous of applying either on their own, or in combination with surgical or radio-therapeutic procedures. Work during the last few years has moved towards (*Table 7*): (*i*) application of the carrier principle and (*ii*) of "latent" activity,

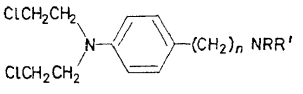
Table 7. Possible attempts at improvement of chemotherapeutic drugs
(From a lecture by F. Bergel at Treatment of Cancer Symposium, Cambridge, 1961)

Chemical and physico-chemical

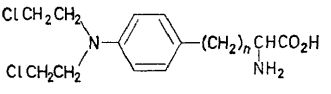
Carrier principle *e.g.* Peptides *etc.*, oligopolymers, carbohydrates *etc.*, lipids	"Latent" activity (local release of active agent)	Utilization of pH-gradients	Selective protection of normal tissues	Selective sensitization of neoplastic tissue

(*iii*) towards utilization of metabolic effects including that of pH gradients and (*iv*) selective protection of normal and selective sensitization of neoplastic tissues *vis-à-vis* drug effects. I can give you only a few illustrations in form of compounds which belong to one or the other group or to several of these groups at the same time.

(*i*) While some workers have utilized amino-acids or peptides as carriers of cytotoxic groups, others have studied sugar-like moieties, pyrimidines, steroids, anti-malarials, *etc.* My colleagues and I have spent a number of years on the study of amino-acid derivatives, especially those based on the naturally occurring phenylalanine and some of its peptides (*Figures 6* and *7*). This research was also taken up, I am glad to say, by Russian scientists under



e.g. CB 3039: $n = 2$; $R = R' = CH_3$



e.g. Melphalan (CB 3025) (L) or sarcolysine (CB 3007) (DL): $n=1$ CB 1385 (DL): $n=2$

Figure 6. Nitrogen mustards with cationic and amino-acid side chains

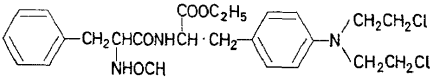


Figure 7. A representative of the melphalan peptide series

Larionov[14], and by Italian and American workers[15]. We have now at our disposal not only the three forms of phenylalanine-*p*-mustard, L = melphalan, D = medphalan and DL = merphalan or sarcolysin, distinguishable by a number of biological tests, *e.g.* on circulating lymphocytes and neutrophils, see Elson *et al.*[16] (*Figures 8a* and *8b*), but also the corresponding *m*- and

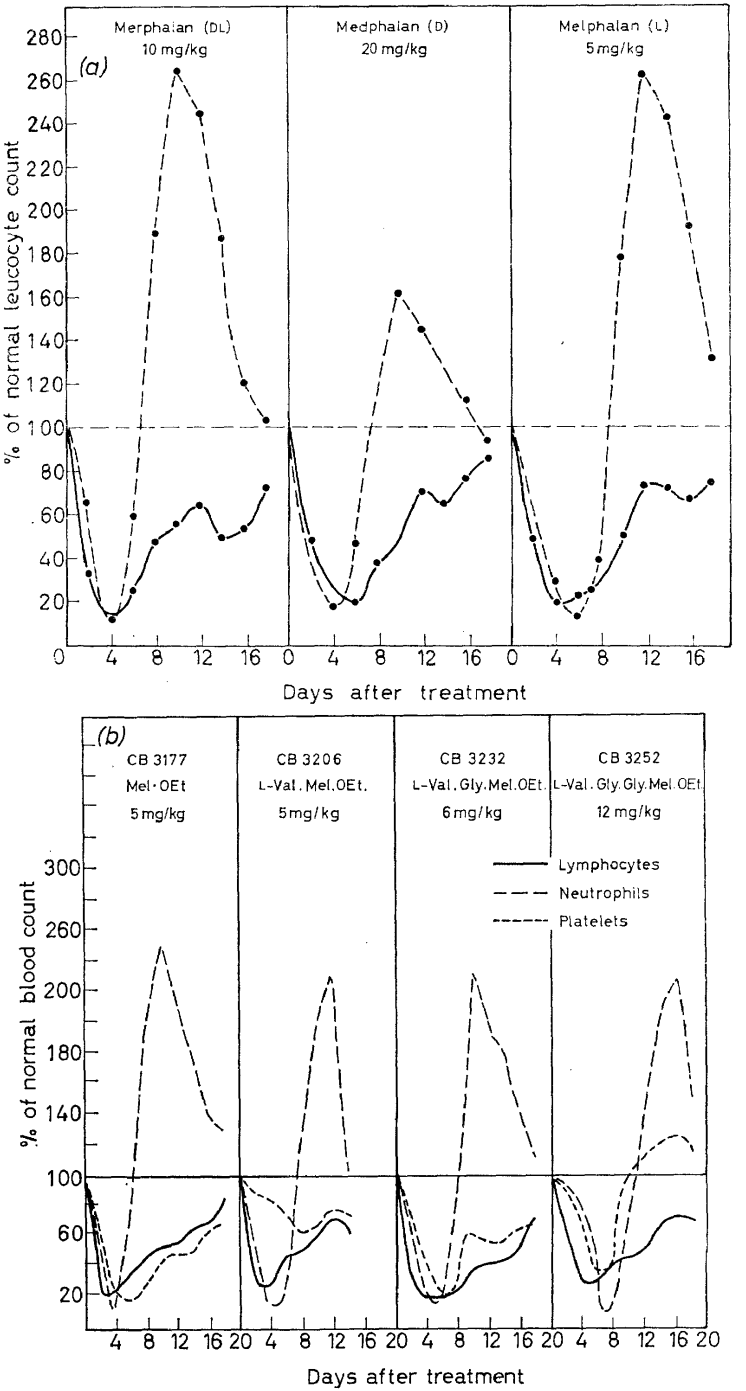


Figure 8. The effect on circulating blood elements of a number of melphalan derivatives
(By courtesy of L. A. Elson *et al. Biochem. Pharmacol.* **11**, 1079, 1962)

o-isomers (merophan)[17] and a number of di- and polypeptides. So far melphalan and sarcolysin have found clinical use, especially in the treatment of malignant melanoma and multiple myeloma; from a fundamental point of view it is not uninteresting that a pentapeptide (CB 3305)[18] with M = 770, has according to Shankman[19], weight for weight, a similar inhibitory effect on the growth of *P. cerevisiae* (*L. citrovorum*) as the parent substance melphalan with M = 305 (*Table 8*).

Table 8. Effect of melphalan and derivatives on micro-organisms

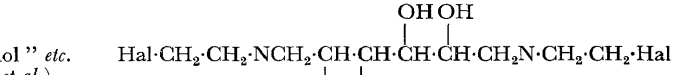
Compound	*50% inhibition of growth P. Cerevisiae = L. citrovorum* (μg/ml)
Melphalan	<200
Pro—gly—val—phe—melphalan ethyl ester hydrochloride	<200
Merophan	No effect at 200
Chlorambucil	No effect at 200
N-Formyl—phe—melphalan	No effect at 200

Tests by Dr. S. Shankman, Pasadena

Another series of cytotoxic drugs with carrier moieties belonging to sugar-like polyols was studied some time ago in Hungary by Varga *et al.* as Degranol and in our Institute by Timmis and Brown[19a] in form of Myleran analogues (*Table 9*). Again, stereospecificity is of importance here in that only

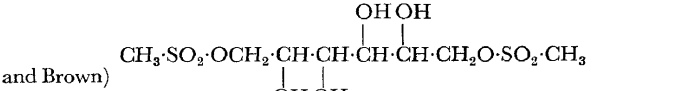
Table 9. Polyol derivatives

" Degranol " *etc.* (Vargha *et al.*)



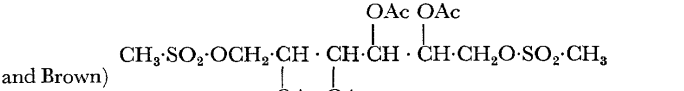
1:6-Di-deoxy-1:6-bis-(2-chlorethylamino)-D-mannitol

CB 2511 (Timmis and Brown)



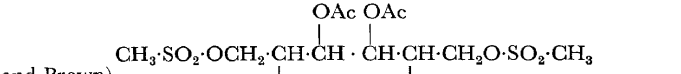
1:6-Di-methanesulphonyl-D-mannitol
250 mg/kg (Walker) C/T ∞ L-mannitol C/T 2·4

CB 2583 (Timmis and Brown)



2:3:4:5-tetra-acetyl-1:6-di-methanesulphonyl-D-mannitol 500 mg/kg (Walker) C/T ∞

CB 2638 (Timmis and Brown)



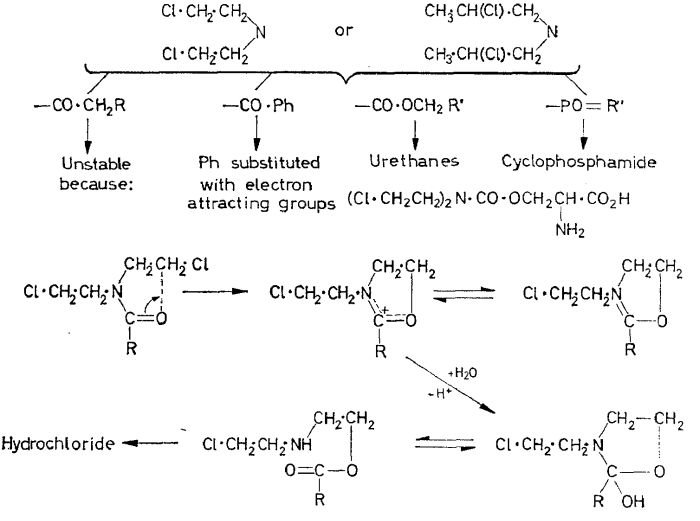
2:3:4:5-tetra-acetyl-1:6-di-methanesulphonyl-dulcitol 500 mg/kg (Walker) C/T 1·45

According to Timmis and Brown (see *Biochem. Pharm.* **3**, 247, 1960)

D-mannitol and L-threitol gave dimethane sulphonates with anti-tumour activity. I shall come back to this further on when discussing utilization of metabolic effects. At this juncture I would like to mention that according to Professor G. Weber, Indianapolis[20], only D, but not L-mannitol myleran and not Degranol, inhibits fructose-1 : 6-diphosphatase and possibly the corresponding phosphorylase.

(*ii*) Going over to "latency", this means compounds carrying deactivated cytotoxic groups which under biological conditions and on release from, or by alteration of, their carrier regain their full destructive effects. It can be achieved among others by combining a N-mustard group in form of an amide with an acid such as carboxylic, carbamic or phosphoric acid. In this manner the alkylating power of dichloroethylamino, in difference to ethyleneimino groups are diminished and the derivatives possess a low chemical and biological activity (*Table 10*). Simple aliphatic amides with a $(ClCH_2CH_2)_2N$ group are not very easily prepared because they rearrange with relatively great facility into esters of hydroxyethylchloroethylamine.

Table 10. " Mustard " carboxamides and phosphoramides

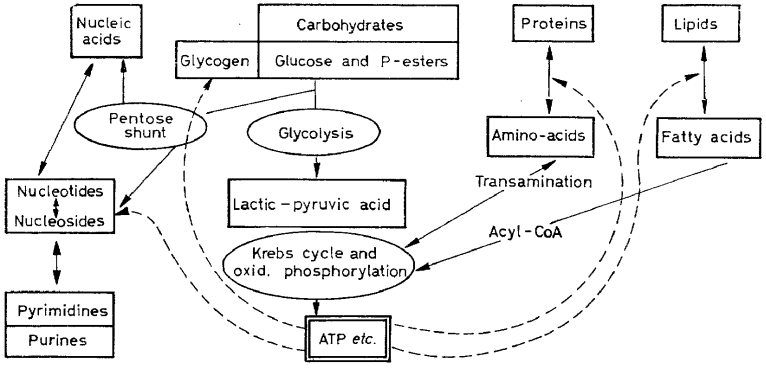


This also happens with the mustard analogues of glutamine and of other amino-acids, so that one has to use a roundabout way: this consists, in the case of serine, of the preparation of the carbamate derivative. One of the phosphoric acid derivatives, Cytoxan (cyclophosphamide), based on the original work of Seligman and Friedman[21] was synthesized and studied by Arnold and Brock *et al.*[22] and introduced into clinical medicine. It appears from recent work in Boston, Mass. that the simple enzymically catalysed detachment of the $HN(CH_2CH_2Cl)_2$ group under biological conditions does not represent fully its mechanism of action; some other intermediate, maybe still carrying the phosphate residue, must be formed and act as cytotoxic agent.

(*iii*) Let me draw your attention briefly to the possibility of utilizing

metabolic effects to improve the action of anti-tumour drugs. The preferential concentration of an anti-tumour drug in the target area may not only be promoted or achieved by the right carrier moiety, by a differential liberation of an active moiety of a "latent" compound as mentioned just now, but also by making use of certain metabolic differences between normal and abnormal tissues, however slight in comparison with the much larger ones between host and invading organisms. One of the claims which has been made again and again since Warburg's discovery of the preponderance of aerobic and anaerobic glycolysis in cancer cells was that protones accumulate either as lactic or pyruvic acid in neoplastic tissue (*Table 11*), and that the acidity of such cells increases with a concurrent fall in pH. This has been

Table 11. Cellular metabolism
(From F. Bergel. *Chemistry of Enzymes in Cancer*, p. 8, 1961. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.)



demonstrated by a number of workers but most persistently by Kahler *et al.*[23] who found considerable changes in pH in tumours after injection of glucose, fructose and in some cases after galactose, very likely measuring these in the intercellular fluid with electrodes (*Figure 9*). So far we could not obtain the same dramatic drop with our rat tumours but glucose and fructose injections appear to increase the anti-tumour effects of basic alkylating agents (*Table 12*) and more so of mannitol Myleran; unfortunately, however, their general toxicity, and to a slightly lesser degree their haemotoxicity (bone marrow damage) go up. Whether these observations support the pH-gradient hypothesis or whether the injected sugars increase the levels of intermediates of the glycolytic pathway making its enzymes, hexokinase, fructose-1 : 6-diphosphatase or phosphorylase more vulnerable, or whether penetration of the drugs into cells and nuclei are favourably influenced, as they may depend on active transport phenomena, we do not know yet. We feel that these observations are worth while pursuing. The selective protection of normal organs with thiol compounds such as cysteine, and the selective sensitization of neoplastic tissue with porphyrins as claimed by various workers[24] and studied by my colleagues has not yet reached the state where, at least in our opinion, we can claim clear cut advances. But our investigations are continued unceasingly to achieve lower toxicities and greater selectivity inside the given limitations.

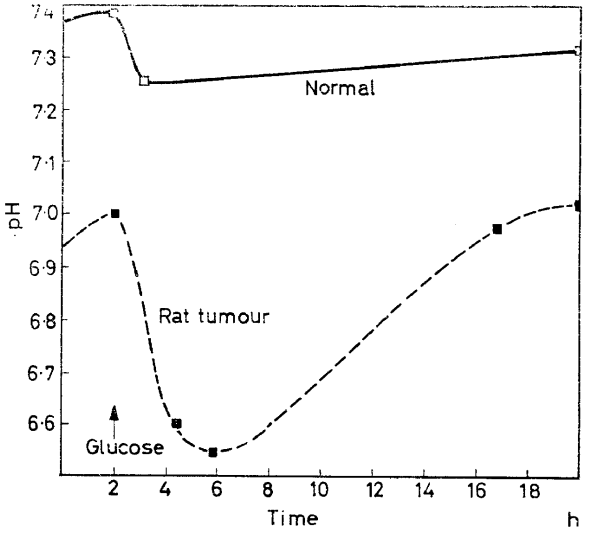


Figure 9. Glucose administration to rats and ensuing effect on pH of normal and tumour tissue
(By courtesy of H. Kahler. *J. Natl. Cancer Inst.* **16**, No. 2, 541, 1955)

Table 12. Glucose effect and basic alkylating agents

Compound	Dose (mg/kg)	C/T ratio	
		Alone	With glucose
CB 1074 M—C$_6$H$_4$— (Ref.12)	20	3·7	3·5 (a) *
CB 1348 M—C$_6$H$_4$—(CH$_2$)$_3$COOH (Ref.13)	1·5	7·2	4·5 (a)
	1·0	1·6	1·0 (a)
CB 3025 M—C$_6$H$_4$—CH$_2$CH(NH$_2$)COOH (Ref.14)	0·5	7·5	7·5 (a)
CB 3177 M—C$_6$H$_4$—CH$_2$CH(NH$_2$)COOEt	0·5	4	18·2 (a)
CB 3039 M—C$_6$H$_4$—CH$_2$CH$_2$NMe$_2$ (Ref.8)	8	8·1	∞ (b)
	8	2·5	16 (a)
Glucose	(a)	0·5	
		0·75	

M= —N(CH$_2$CH$_2$Cl)$_2$
(a) 5g/kg of glucose at −1·0, and 1h after drug
(b) 5g/kg of glucose at −2,−1,0,1, and 2h after drug
(c) 7·5g/kg of glucose at −1·0, and 1h after drug

(3) At this point I should mention the group of metabolic antagonists or enzyme inhibitors which have proved their value in carcino-chemotherapy since Farber *et al.*[25] in 1947 used a folic acid analogue for the first time. But time is too short to deal with them properly. I hope to have your consent to treat briefly instead more speculative aspects and leave aside anti-amino acids, anti-vitamins (anti-coenzymes), and pyrimidine, purine and folic acid analogues, recent studies of which have given an insight into their mechanism of action and of specific drug resistance. The speculative approach refers to two working hypotheses; their final usefulness can only be judged by their future practical successes. One is being developed by Baker *et al.*[26] in the U.S.A., the other by my colleagues and myself in London. Baker, starting from the assumption that irreversible inhibitors of essential enzymes may be more effective than reversible and competitive ones, is studying the combination of the classical structure of the latter type, which fits on to the catalytic site, with a group producing covalent bonds by endo- or exo-alkylation or acylation. This work aiming at an extension of the antagonist idea by non-classical means may produce, later, interesting drugs. (*Figure 10*).

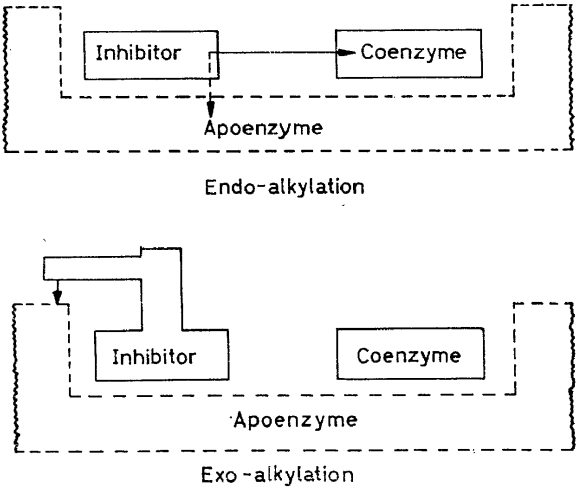


Figure 10
(By courtesy of B. R. Baker. *Cancer Chemotherapy Repts.* No. 4, 1, 1959)

Our scheme is probably just as speculative and ambitious. It rests on three assumptions: one, is it possible to re-introduce into tumour cells certain functional constituents of which these tumours are deficient; two, is it useful to raise levels of enzymes which regulate the catabolism of intermediates on which the tumour cell depends more than the normal one; and three, could all this be achieved with enzyme models, or as I called them, "enzymomimetics", that is relatively simple compounds or compound combinations, capable of imitating under *in vivo* conditions the true biocatalysts. Some of you may remember that a number of workers including ourselves have found effects of purified enzyme preparations, such as xanthine oxidase or ribonuclease, on the growth rate of a spontaneous mammary tumour in the

mouse[27]. Other workers succeeded in diminishing ascites tumours with DNAase[28] and asparaginase; enzymes, such as lipase, proteinases *etc.* have been tested from time to time, especially on tissue cultures. But with all these natural materials the problem remains whether these large molecules, often from a different species, ever penetrate the target cells sufficiently and without immunological reactions. This is why we started to look for enzyme models to replace the natural products. One of the examples I wish to give you is that of a model for cysteindesulphydrase. It would take a whole lecture of its own to explain the why and wherefores[29], but may it suffice to say that there is a possibility that human leukaemic cells are more avid for sulphur-containing amino-acids such as cystine and cysteine (according to Weisberger[30]) than normal leucocytes and that a combination of pyridoxal phosphate and VO^{2+} (pyrvanal) was found by my colleague Harrap[31] to act as a cysteine-destroying agent *in vitro* and *in vivo* (*Figures 11* and *12*). Under *in vivo* conditions this expressed itself in an inhibition of fowl

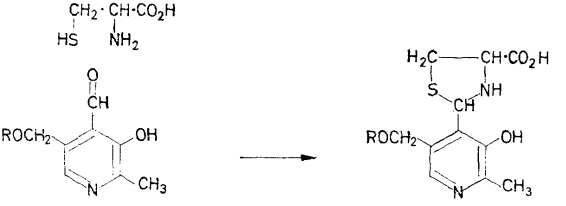


Figure 11. Cysteine and pyridoxal derivatives
(From F. Bergel. Chemistry of Enzymes in Cancer, p. 77, 1961. Courtesy of Charles C. Thomas, Publishers, Springfield, Illinois)

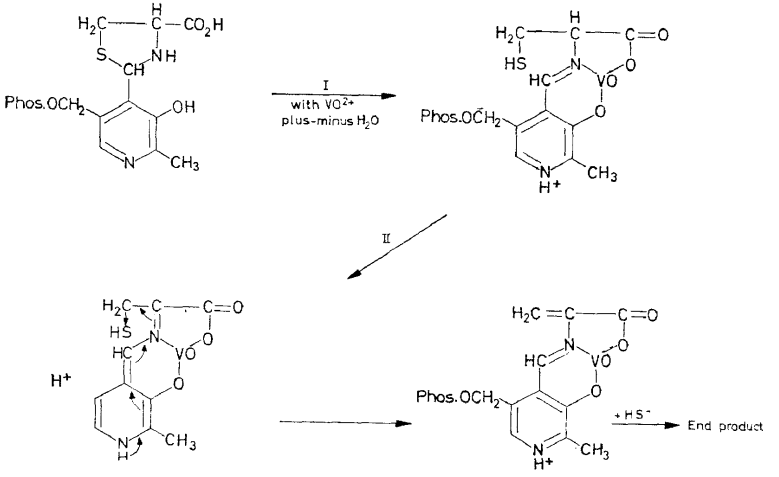


Figure 12. Mechanism of pyrvanal action
(By courtesy of the Chemical Society from F. Bergel *et al. J. Chem. Soc.* **1962**, 1101)

tumours implanted in the chorioallantoic membrane of the chicken egg (*Table 13*) and in a fall of the white blood cell count in, so far, a very few patients with acute leukaemia.

What we need to establish now is the capacity of such patients when

Table 13. Average weights of embryos and tumours in pyrvanal chemotherapy of fowl sarcomas (expressed as percentage of control values)[29]

	DR"D"				*Rous*		
	Control	*Pyrvanal*	*Pyridoxal phosphate alone*	NH_4VO_3 *alone*	*Control*	*Pyrvanal*	NH_4VO_3 *alone*
Embryos	100	79	82	100	100	100	96
Tumours	100	55	78	100	100	47	70

Dose: Pyridoxal phosphate 1·11 mg, Ammonium vanadate 0·04 mg } in 0·25 ml water
Controls 0·25 ml physiological saline

given vitamin B_6-tablets and sodium vanadate solution to effectively transform B_6 into pyridoxal or pyridoxal phosphate so that the metal ion and the pro-coenzyme could diminish the cysteine pool. Work in this direction is being carried on in our Institute.

Such work in human pathological chemistry, and I mentioned before the desirability of assessing differences in biochemical functionalities between tumour and tumour, and tumour and normal tissue, is of vital importance. An enormous number of investigations have been carried out over the last 30 years or so on animal material. But there is great necessity to concentrate the resources and researches in many places on human cancers and leukaemias and to find out, apart from quantitative differences in cellular constituents (enzymes, nucleic acids, *etc.*) and in metabolic rates, much more about the behaviour of human tissues *vis-à-vis* drugs. This could be done in a number of ways, and at this point I can give you only a few examples. Some tumour samples from biopsies and major operations can be transformed into primary tissue cultures (see Ambrose *et al.*[32]) which then can be exposed to various concentrations of a number of anti-tumour agents (*Figure 13*). The LD_{50} of the latter can be established and their effectiveness

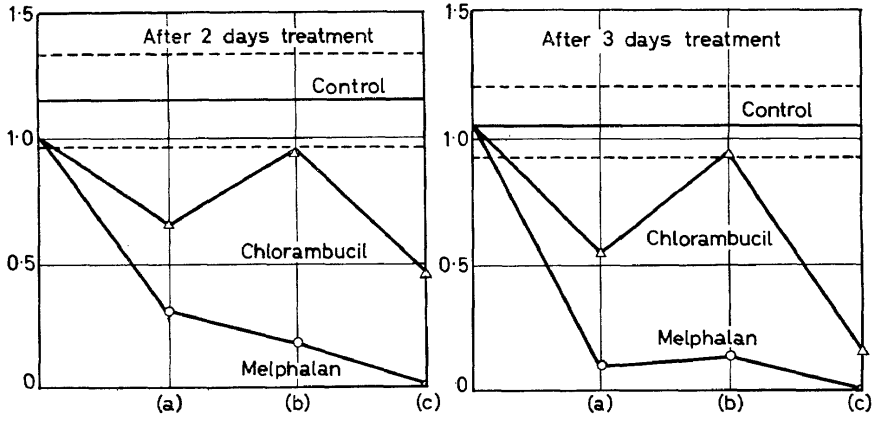


Figure 13. Effect of melphalan and chlorambucil on human Melanoma cultures
(By courtesy of E. J. Ambrose *et al. Lancet* i, 24, 1962)

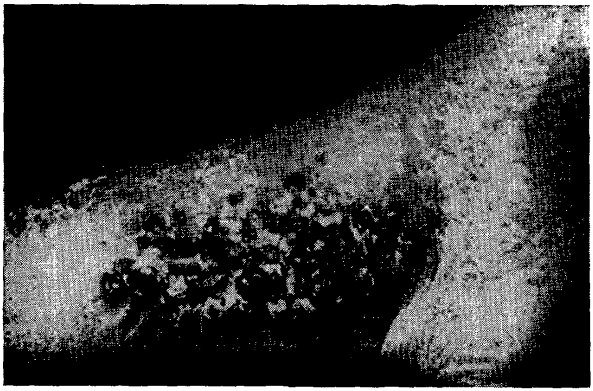


Figure 14(a). Malignant melanoma

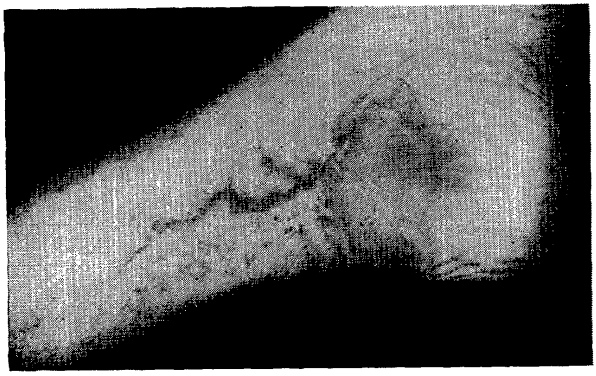


Figure 14(b). Malignant melanoma treated with melphalan during regional perfusion

(By courtesy of O. Garai, C. Cooling, and D. Staunton. *Brit. Med. J.* i, 1231, 1962)

assessed. In the case of a human malignant melanoma there appeared to be a difference in cytotoxic activity between otherwise closely related alkylating agents (melphalan and chlorambucil and thioTEPA).

Another approach consists in determining after Wolberg[33], the uptake of tritium-labelled uridine into tumour cells before and after exposure to chemotherapeutic agents. The amount of radioactive material is then estimated with the help of autoradiography of the tissue sample and the damage to the nucleic acid anabolizing systems assessed. Again, other techniques would deal with the rate of glycolysis and respiration prior and subsequent to the application of drugs. Only if all this and many more investigations are carried out and, if possible, brought to the stage of a semi-routine test, shall we understand why certain tumours in certain patients respond to chemotherapy and others do not, why after successful first courses the tumours become resistant and why some primary cancers spread while others are held in check. In addition, any attempt of the surgeons and radiotherapists to come to a collaborative arrangement with the chemotherapist should be strongly supported by the latter and any help required of him given generously. Then we might see more than a relatively small, relatively short-lived number of clinical successes, one of which I want to show you as a last illustration (*Figures 14(a)* and *(b)*). Here is a patient with malignant melanoma prior to and after regional perfusion with melphalan. Such is the situation that we are most encouraged for our further labours by successes of this nature.

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