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### Clinical Trials of a Chemical Agent, in Conjunction with X-ray Therapy, and Related Laboratory Studies (J. S. Mitchell)

An attempt is being made to improve the results of the radiotherapy of some types of cancer by the ancillary use of chemotherapeutic agents, designed to modify the effects of radiation on the metabolism of cells.

Clinical trials of large doses of tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate (Synkavit), mainly in conjunction with palliative X-ray therapy in patients with various types of advanced malignant tumours have been in progress since November 1946 (Mitchell and Simon-Reuss, 1947 ; Mitchell, 1948 and 1949). The following information may be added to that given in the last Report.

The general survey of the treatment of patients with advanced malignant tumours continues and in all 511 cases have been treated by the combination of X-ray therapy and the compound. The general survey includes almost all those cases of carcinoma of the mouth (excluding lip), pharynx, larynx, nasal sinuses and oesophagus, for which surgery is not the main method of treatment, and the majority of inoperable cases of malignant tumours of other types but with the exception of all tumours in infants and young people. Interesting results have been obtained in some cases of carcinoma of the tongue, extrinsic carcinoma of the larynx, malignant tumours of the ovary and advanced carcinoma of the breast. There is some evidence that the compound may produce re-radiosensitisation in some cases of advanced carcinoma of the skin which have become radioresistant after much previous irradiation. It is too early to make any final assessment.

It is considered that the compound is best administered by repeated daily intravenous injections. The dosage now normally used for intravenous injection is to start with 25 mg. increasing in steps of 25 mg. daily to 100 mg. For most patients the best daily dose for intravenous injection appears to be 100 mg. Many patients will tolerate 150 mg. ; a few will tolerate 250 mg. daily. Occasionally larger daily doses, *e.g.* 500 mg. have been given. It is often necessary at the higher dosage levels to reduce the daily dose after a time. The largest total doses administered in a course of intravenous injections have been 7,310 mg., given in 19 injections in an overall time of 26 days, and 8,140 mg., given in 31 injections on consecutive days. It seems best to start the injections at least 5 days before the X-ray treatment and to try to arrange that the daily injection is given half and one hour before the individual X-ray treatments. The injections are usually continued until the course of X-ray treatment is completed, but recently an attempt has been made to continue the administration of the compound until the radiation reaction has finished.

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For intramuscular injection the unit daily dose has generally been 100 mg. but larger doses, *e.g.* 500 mg. daily for 42 days have been given without any demonstrable toxic effects. Oral administration of *e.g.* 100 mg. tablets 3 times daily has been used especially in the treatment of out-patients, but its value is as yet uncertain.

The low toxicity of the compound has been confirmed. After intravenous injection there is often transient flushing and sometimes nausea, vomiting and faintness especially after doses larger than 200 mg. The larger intravenous doses produce almost immediate focal pain or focal sensations in the region of the tumour in many cases, especially where there is involvement of bone. Focal reactions were observed in 122 out of 182 cases receiving intravenous injections and often were the factor which limited the dose. After intramuscular injections, focal reactions may occur but are much less frequent.

## Treatment of Inoperable Carcinoma of the Bronchus

The results of the special study of the effects of treatment on time of survival of inoperable cases of cancinoma of the bronchus may be summarised as follows :—

The "control" group was treated by X-ray therapy from 1944 to May, 1947. The "treated" group was treated by X-ray therapy combined with the compound, tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate, administered by intramuscular and/or intravenous injection from May, 1947 to December, 1949 inclusive.

Cases with complete histological verification, mainly at bronchial biopsy and post-mortem

Control group : X-ray therapy only.

19 cases : Mean survival 3.89 months from first X-ray treatment and 11.00 months from first symptom.

Only one case (which survived 15 months) survived more than 8 months from first treatment and 3 cases survived more than 15 months from first symptom.

Treated group : X-ray therapy combined with the compound.

29 cases : Mean survival 9.38 months from first X-ray treatment and 17.55 months from first symptom.

Of these, 15 cases survived more than 8 months from first treatment and 15 cases also survived more than 15 months from first symptom.

The survival time from the first X-ray treatment is significantly greater in the "treated" group than in the "control" group ($n=46$, $t=3.519$, $P=0.001$). Even if the cases surviving only one month from the first treatment are excluded, to meet the criticism of a possible difference in the selection of cases, the difference in survival between the treated and control groups can still be regarded as significant

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($n=39$, $t=2.457$, P is slightly less than 0.02). The survival time from the first symptom is regarded as significantly greater in the treated group than in the control group, ($n=46$, $t=2.624$, $P=0.012$).

Within the treated group, 20 cases were treated by X-ray therapy combined with the compound administered by intravenous or by intramuscular and intravenous injection. The survival time from the first X-ray treatment in this treated sub-group was 11.05 months and is greater than that in the control group ($n=37$, $t=4.345$, $P<0.001$).

There is suggestive evidence that X-ray therapy combined with intravenous compound is associated with longer survival from the first treatment (11.17 months) than is X-ray therapy combined with intramuscular compound (6.45 months) ; ($n=27$, $t=2.106$, $P<0.05$).

The dosage levels used in the X-ray therapy were not appreciably different in the control and treated groups. Of the former, 4 cases received radical X-ray therapy and of the latter 3 received radical X-ray therapy ; all the other cases received palliative X-ray therapy. There was no relevant difference in the distribution of the histological types of growth in the control and treated groups.

All confirmed cases, including, in addition, those cases :—

(*a*) not verified histologically but with typical bronchoscopic appearances together with metastases at some stage, and those with,

(*b*) positive histology on metastases in supraclavicular glands, skin or elsewhere but no bronchoscopic examination, or bronchoscopic appearances not definite.

Control group : X-ray therapy only.

34 cases : Mean survival 3.82 months from the first X-ray treatment and 10.00 months from first symptom.

Treated group : X-ray therapy combined with the compound.

47 cases : Mean survival 7.81 months from first X-ray treatment and 14.53 months from first symptom.

The survival time from the first X-ray treatment is significantly greater in the "treated" group than in the "control" group ($n=79$, $t=3.630$, $P<0.001$). The survival time from the first symptom is also to be regarded as significantly greater in the treated group than in the control group ($n=79$, $t=2.678$, $P<0.01$).

From these results, it is concluded that in the treatment of inoperable cases of carcinoma of the bronchus by X-ray therapy, the ancillary use of the compound, tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate, produces a small increase in the length of survival. This finding is in accordance with clinical impressions and the increased survival appears to be associated with useful life. Details of this investigation will be published elsewhere.

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## Laboratory Studies

The investigation of mitotic inhibition and cell degeneration produced in tissue cultures of chick fibroblasts by chemical agents, alone and in combination with X radiation has been continued, in collaboration with Mrs. I. Simon-Reuss, of this Department.

Further studies of the dependence of mitotic inhibition upon concentration have been made in the case of tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate (Synkavit). A linear relation was found between the mitotic inhibition after 24 hours, expressed in probits, and the logarithm of the molar concentration of the chemical. Under the experimental conditions, 50 per cent. mitotic inhibition was produced by the concentration of $3.94 \pm 0.74 \times 10^{-6}$M.

A number of new compounds has been tested to study the relation between antimitotic activity and chemical structure. These include the following compounds, which were kindly given by Dr. F. Bergel :—

(i) Di-calcium salt of 2:3:6-trimethyl-hydroquinone diphosphate, which produced 50 per cent. mitotic inhibition at approximately $6 \times 10^{-6}$M. concentration.

(ii) Pentasodium salt of 1:4-dihydroxy-2-naphthoic acid diphosphate, which gave 50 per cent. mitotic inhibition at $5 \times 10^{-6}$M.

(iii) Hexasodium salt of 1:4-dihydroxy-2:3-naphthalene dicarboxylic acid diphosphate, which was less active than (ii), producing 50 per cent. mitotic inhibition at $9 \times 10^{-6}$M.

It is of interest in relation to the mechanism of the antimitotic action of tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate that compound (i) in which the second ring is replaced by the 2 methyl groups is only slightly less active, and further, it is slightly more active than toluhydroquinone diphosphate, and that compound (ii) in which the methyl group is replaced by carboxyl is only very slightly less active. Introduction of the second carboxyl group in the 3-position giving compound (iii) somewhat reduces the antimitotic activity.

Among the simpler compounds, *p*-nitrophenyl phosphate produced 50 per cent. mitotic inhibition at a concentration of $4 \times 10^{-6}$M. ; the introduction of the *p*-nitro group thus reduced the antimitotic activity by a factor of four.

As an additional method of approach, the mechanism of action of tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate has been studied in chick fibroblast cultures from the point of view of antagonisation of the antimitotic effect by nucleotides, nucleosides, purines and pyrimidines and other related compounds. In most cases, the compounds were used in concentration $5 \times 10^{-6}$M. ; parallel experiments have been started to study possible antagonisation of the effects of a small dose of X radiation (300 r).

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The results obtained so far under the experimental conditions using chick fibroblast cultures may be summarised as follows :—

(A) The mitotic inhibition produced by tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate is abolished by the following compounds which by themselves in the concentrations used have no inhibiting effect on mitosis :—

(*a*) Hypoxanthine.

(*b*) Adenosine.

(*c*) Guanylic acid, which increases the mitotic count.

(*d*) Cytidylic acid.

(B) The mitotic inhibition produced by tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate is abolished by compounds which alone are mitotic inhibitors, *e.g.* guanosine.

(C) There is at least partial additivity of the mitotic inhibition produced by tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate and the following added compounds :—

(*a*) Yeast adenylic acid.

(*b*) Isoguanine riboside (the specimen of this compound was kindly given by Professor A. R. Todd).

(D) The mitotic inhibition produced by tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate is unchanged by the following compounds which are not mitotic inhibitors by themselves in the concentrations used :—

(*a*) Uracil.

(*b*) Muscle adenylic acid.

(*c*) Folic acid.

The combination of tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate and guanylic acid, which has no mitotic inhibiting effect, with 300 r of X radiation produces a very high proportion of cells with mitotic abnormalities including anaphase bridges, chromosome fragments, clumped metaphases, gross pycnoses and exploded cells. Much further work in this field is required.

The occurrence of focal pain and sensations in human tumours after intravenous injection of the compound, tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate, has been found to be associated with increased fluorescence of the tumour in some cases and the time relations of this fluorescence have been measured with the aid of a photomultiplier. Animal experiments using the Walker rat carcinoma have confirmed the increased fluorescence of the non-necrotic parts of the tumour and of some other tissues including lymphoid tissue after intramuscular injection of the compound. The appearance of the yellow fluorescence induced by ultra-violet light is delayed. Measurements of the spectrum of the fluorescent light are in progress.

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**Effect of X Rays on Protein Synthesis** (B. E. Holmes)

The work described in the last Report has been continued.

"Tagged" amino acids (glycine containing $N^{15}$ and methionine containing radioactive sulphur) have been used to follow the synthesis, or turnover, of the cell proteins of the Jensen rat sarcoma in normal conditions and after X rays. The protein fractions used were, as before, the histone extracted from the nuclei, the ribonucleoprotein of the cytoplasm and the remaining heat-coagulable proteins of the cytoplasm.

The results with methionine confirm those obtained with inorganic $S^{35}$ and described in the previous Report. No constant effect of X rays can be seen in the amino-acid turnover of any of the protein fractions. In some experiments the synthesis of the nucleic acids (using $P^{32}$ as tracer) and the synthesis of the protein fractions (using methionine $S^{35}$) were followed in the same animal. A repetition of the counting after 14 days showed that the $P^{32}$ and $S^{35}$ had been successfully separated. These experiments showed that there is no relationship between the effect of X rays on nucleic acid synthesis and on the turnover of the histone of the nucleus or of any other protein fraction studied.

Controls were carried out to ensure that the methionine found in the histone fraction was not merely absorbed on the protein. Since the nucleohistone from the thymus has been shown to contain very little sulphur-containing amino acid (Davidson, 1950 ; Allgén, 1950) it has been specially necessary to demonstrate that the activity found by us is not contained in any contaminant of the histone fraction. For this purpose we are using a paper chromatographic technique developed by Mr. N. W. Rees (of the Sir William Dunn Institute of Biochemistry) during his work with salmine and insulin, and are most grateful for his help. All the later part of this work, which will be published shortly, has been carried out in collaboration with Miss L. K. Mee.

The experiments with glycine $N^{15}$ are not quite complete, but also indicate that there is no constant effect of X rays on protein turnover.

The possibility that there may be a lack of correspondence between histone and nucleic acid synthesis after X rays, must be borne in mind. The work of Allgén (1950) describes the physical changes produced in thymus nucleoprotein by gradual removal of the nucleic acid or of the protein part.

**Effect of Various Agents on Nucleic Acid Synthesis** (B. E. Holmes)

The most recent work we have done on nucleic acid synthesis (using $P^{32}$) has demonstrated that irradiation of the tumours *in vivo* with 2,000 r causes an inhibition of roughly 50 per cent. in the synthesis of thymonucleic acid and no effect on the ribonucleic acid turnover or $P^{32}$ uptake into the cell. The temporary effect previously seen, suggesting a much greater effect on thymonucleic synthesis and some inhibition of ribonucleic acid turnover, we have concluded must have been in part due to some shock produced by the fastening of the animal during irradiation. Professor H. N. Green and co-workers (1949) had shown that shock could produce

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inhibition of mitosis in normal tissue, and were kind enough to allow us to follow the uptake of $P^{32}$ into rat tumours following shock. This work is in a preliminary stage, but indicates a large inhibition of ribonucleic acid turnover, thymonucleic synthesis and uptake of $P^{32}$ into the cell. (This last effect had been noticed to a smaller extent by Hevesy (1945) and by myself (Holmes, 1949). It may be supposed that this more general effect of shock on phosphorylating reactions occurred to a slight extent in our earlier experiments and, during the first hour after irradiation, was added to the, much more specific, effect of 2,000 r X radiation just described.

Histological studies of these tumours after shock are in progress and also histological studies of tumours in which the ribonucleic turnover and thymonucleic synthesis has been inhibited by Nembutal (Holmes, 1947).

It is hoped that by comparing the histological picture with the rate of nucleic synthesis after these and other effective agents, we may obtain some evidence as to the time relationship between nucleic acid synthesis and mitosis.

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Reprint from 28th
Annual Report of
the British Empire
Cancer Campaign.
pp.213-219(1.10.1951)

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