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Pretherapeutic Detection of Tumour Resistance and the Results of Tumour Chemotherapy

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Abstract—*A short-term* in vitro *test is described which can be used to detect proliferation-dependent and induced tumour resistance to cytostatic agents. The test involves incubation of tumour cell suspensions with cytostatic agents for short periods. The resulting effects on nucleic acid metabolism are measured using radioactively labelled precursors of DNA or RNA.*

The Walker carcinosarcoma and a neurosarcoma respond differently to treatment with adriamycin, in accordance with the relative growth rates of the tumours. This differential sensitivity of the tumours to adriamycin can also be detected in the test. Similarly, an induced resistance to adriamycin which was developed in the Sarcoma 180 *was also found using the test, as was cross resistance to other cytostatics. Human lung and ovarian carcinomas which were only slightly inhibited by adriamycin* in vitro *before starting treatment* ($<30\%$ *inhibition at a concentration of* 10^{-2} mg/ml *or* 1.7×10^{-5} M) *proved to be resistant to chemotherapy in the clinic. Tumours which showed a clear response to adriamycin in the test* ($>30\%$ *inhibition*) *showed in most cases remission on clinical treatment with cytostatics. A clear correlation was observed between the inhibitory effects of adriamycin* in vitro *and the course of clinical therapy even when a combination therapy was used which did not include adriamycin. A significant correlation was also obtained between the inhibition due to adriamycin* in vitro *and the survival times of the tumour-bearing patients. The short-term test can therefore be used to distinguish between chemoresistant and chemosensitive tumours.*

INTRODUCTION

MALIGNANT tumours respond in individual ways to treatment with antineoplastic substances. Some tumours respond well to chemotherapy, whereas others show only a very weak reaction or even demonstrate resistance to the drug used. Many tumours react to treatment with cytostatics according to their proliferative activity [1]. Tumours with a low rate of proliferation very often show no reaction to treatment (primary resistance), whereas strongly proliferating tumours usually respond to therapy. Furthermore, tumours which respond well to treatment with cytostatics can lose their sensitivity to the drug used during the course of treatment (acquired or induced resistance).

The consideration of both primary and induced resistance is important in chemotherapy. The existence of primary resistance could mean that the use of chemotherapy in general is questionable, and induced resistance would indicate that treatment with the particular cytostatic agent should be discontinued [2]. It would therefore be of great value to individual patients if it could be established, before commencing treatment with antineoplastic substances, whether the tumours would respond to the proposed therapy. If the tumour was resistant, the therapy would only give rise to toxic effects in various normal tissues without having any influence on the tumour growth. With a view to detecting resistance of tumours to chemotherapy before commencing treatment, various test systems have been developed over the past few years

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in order to measure the sensitivity or resistance of malignant tumours [3–13], (for review, see [14, 15]). The possibility that such tests might also be carried out in the clinic was opened by the development of a simple method involving short-term incubation of native tumour material, in the form of isolated tumour cells, and the determination of the effects of added cytostatic agents on the incorporation of radioactive precursors into nucleic acids [2, 16–21]. The usefulness of any method for testing tumour resistance is, however, dependent on its ability to predict the results of therapy. In the present studies, the ability of the short-term test to detect proliferation dependent and induced adriamycin-resistance in tumours was investigated.

MATERIALS AND METHODS

Animal tumours

The transplanted tumours used were the solid Walker carcinosarcoma and a solid neurosarcoma [22], both grown on Sprague–Dawley rats (200 g, male, obtained from Musrattus, Ltd., Brunnthal/Munich), and the mouse Sarcoma 180 which was grown in ascites form in NMRI mice (25–30 g, female, obtained from Ivanovas, Ltd., Kisslegg). The tumours were transplanted at the following intervals: Walker carcinosarcoma (ascites) 3 days; neurosarcoma (solid) 21 days and Sarcoma 180 (ascites) 7 days. The animals were maintained under standard conditions (Altromin® standard diet, Altrogge, Ltd., Lage/Lippe, and water was available *ad libitium*).

Human tumours

Inoperable human lung tumours and ovarian carcinomas were checked histologically. Bronchial carcinomas were obtained from the Thoraxchirurgische Spezialklinik in Heidelberg-Rohrbach (Director: Prof. Dr. I. Vogt-Moykopf) and the ovarian carcinomas from the Universitäts-Frauenklinik in Heidelberg (Director: Prof. Dr. F. Kubli).

Cytostatics

Adriamycin (Adriablastin®, Farmitalia, Freiburg i.Br.); cyclophosphamide (Endoxan®, Asta, Brackwede); 4-hydroperoxy-cyclophosphamide (We thank Prof. Hohorst, Abt. für Zellchemie im Klinikum Frankfurt, Main, for providing this substance, which is an *in vitro* active derivative of cyclophosphamide); daunomycin (Daunoblastin®, Farmitalia, Freiburg i.Br.); 5-fluorouracil (Fluoruracil-Roche, Hoffmann–La Roche, Grenzach; methotrexate (Lederle-Cyanamid, München); vincristine (Lilly, Gießen).

Radioactive substances

(6–^{3}H)-thymidine (spec. act. 20–30 Ci/mmole, TRK 61), (G–^{3}H)-uridine (spec. act. 2–10 Ci/mmole, TRK 27) and deoxy-(^{3}H)-uridine (spec. act. 10 Ci/mmole, TRK 242) were obtained from Amersham Buchler GmbH, Braunschweig.

Development of an adriamycin resistant tumour cell line from the Sarcoma 180

Ascites tumour cells were treated with adriamycin (3×0.5 mg/kg body weight per week) during 25 passages.

Short-term test

The tumour material was freed from fat and muscle tissue and necroses. It was then placed in plastic Petri dishes and cut into small pieces with scissors under sterile conditions. The tissue pieces were suspended in Hanks solution, pipetted several times into a sharp-edged glass pipette and the resulting cell suspension filtered through gauze (pore size 200 μm). The cells were collected in culture tubes, centrifuged for 5 min at 200 ***g*** and washed with Hanks solution. After centrifugation the Hanks solution was decanted and the pellet resuspended in TCM-199 (Difco). The cell count was adjusted to 500,000 cells/ml using a Neubauer counting chamber. In the case of non-sterile tumours, a broad spectrum antibiotic (Cephalotin®, Lilly, 50 μg/ml) was added to the cell suspension. The cells were then distributed into test tubes in aliquots of 0.9 ml. After pre-incubation at 37°C for 15 min in a shaking water bath, the cytostatics were added to the test-suspensions (50 μl/test tube). The concentrations used of adriamycin, daunomycin, 5-fluorouracil and 4-hydroperoxy-cyclophosphamide were: 10^{-1}, 3×10^{-1}, 10^{-2}, 3×10^{-2}, 10^{-3}, 3×10^{-3} and 10^{-4} mg/ml. The cytostatics were dissolved in TCM-199 prior to addition. After incubation for 2 hr, the radioactive nucleic acid precursors were pipetted into the test tubes (2.5 μCi/ml cell suspension). The appropriate radioactive nucleic acid precursor was used for each cytostatic agent [19]. ^{3}H-uridine was used for both adriamycin and daunomycin, whereas ^{3}H-thymidine and ^{3}H-deoxyuridine

were used for 4-hydroperoxy-cyclophosphamide and 5-fluorouracil respectively. Incubation was continued for 1 hr, after which 100 µl aliquots were pipetted from each test tube on to round filter papers (Whatman Filters: 3MM, 2.3 cm, Balston Ltd., England) and dried in a stream of warm air. The non-incorporated radioactivity was extracted with ice-cold 5% trichloroacetic acid (TCA) (twice for 30 min; 100 filters to 1 l TCA). The filters were then washed in ethanol:ether (1:2, 20 min) ether (10 min) and air-dried. Five milliliters scintillation fluid (85 ml Szintol 3, Koch-Light Laboratories Ltd., England, to 2.5 l toluene) was added to each filter in a scintillation vial and the incorporated radioactivity in counts/min determined by liquid scintillation counting [16, 17]. The cell suspensions were examined microscopically during the incubation period. Furthermore the kinetic of incorporation of radioactive precursors was determined in each test.

Application of cytostatic agents and evaluation of the results of therapy

Animal tumours, (*a*) *solid.* As soon as the solid tumours had reached a measurable size (Walker carcinosarcoma: 3–4 days, neurosarcoma: 2–3 weeks after subcutaneous transplantation of the tumours under the back skin) the cytostatics were injected i.p. and the tumour size measured using a micrometer gauge (2 diameters). The average daily tumour growth was calculated for the Walker carcinosarcoma from the difference in tumour size between the 1st and 4th days after starting treatment. For the neurosarcoma, tumor growth was calculated from the size difference between the 1st and 7th days.

(*b*) *Ascites.* The test substances were injected i.p. on the 3rd and 5th days after transplantation of the tumours. The cytostatic effect was measured on the 7th day after transplantation by determination of the cell count using a Coulter counter (Coulter Electronics, Ltd.).

Human tumours, (*a*) *lung carcinomas.* A total of 40 patients with inoperable tumours were treated with cytostatic agents between October 1973 and May 1975. Of these, 15 were treated only with adriamycin (group A) [23] and 10 received therapy using a combination of adriamycin with either vincristine alone or together with cyclophosphamide or methotrexate (group B) [24]. Fifteen further patients received combination therapy without adriamycin, using either vincristine-cyclophosphamide or vincristine-cyclophosphamide-5-fluorouracil-methotrexate (group C) [25].

(*b*) *Ovarian carcinomas.* Therapy of patients with ovarian carcinomas (FIGO stages III/IV) consisted either of pulse therapy with cyclophosphamide followed by long-term therapy with cyclophosphamide and gestagen [26] or of combination chemotherapy involving cyclophosphamide-fluorouracil according to the data of Senn *et al.* [27]. In cases where patients failed to respond to this therapy, a combination of vincristine and adriamycin was applied [28].

The evaluation of the success of therapy was carried out according to the guidelines of the Schweizerischen Arbeitsgemeinschaft für klinische Krebsforschung (SAKK, [29, 30]). Remission was defined as the measurable regression of tumour size after treatment with cytostatic agents, as estimated from X-ray photographs (lung carcinomas) or palpation (ovarian tumours). Progression was defined as the measurable increase of tumour-size during therapy. The survival times were measured from the time at which the tests were carried out.

RESULTS

Animal tumours

The faster the tumour growth, the stronger is the effect of the drug [2, 17, 31, 32]. The Walker carcinosarcoma and the neurosarcoma provide suitable models for the therapy of rapidly growing and slowly growing tumours respectively. If no therapy is carried out, rats with the Walker carcinosarcoma survive for 10 days and those with the neurosarcoma for 10 weeks. The actively growing fraction of the tumour is about 60% of the Walker carcinosarcoma but only 15% of the neurosarcoma. The tumours respond to treatment with cytostatics in a manner which is dependent on their respective growth rates. For example, adriamycin has only a weak effect on the the neurosarcoma (Fig. 1, left), whereas the growth of the Walker carcinosarcoma is appreciably inhibited by the same concentration of adriamycin. This differential proliferation-dependent sensitivity of the tumours to adriamycin is also observed in the *in vitro* short-term test (Fig. 1, right). The incorporation of ^{3}H-uridine in tumour cells from the Walker carcinosarcoma (● ▲) is more strongly reduced by adriamycin than in the

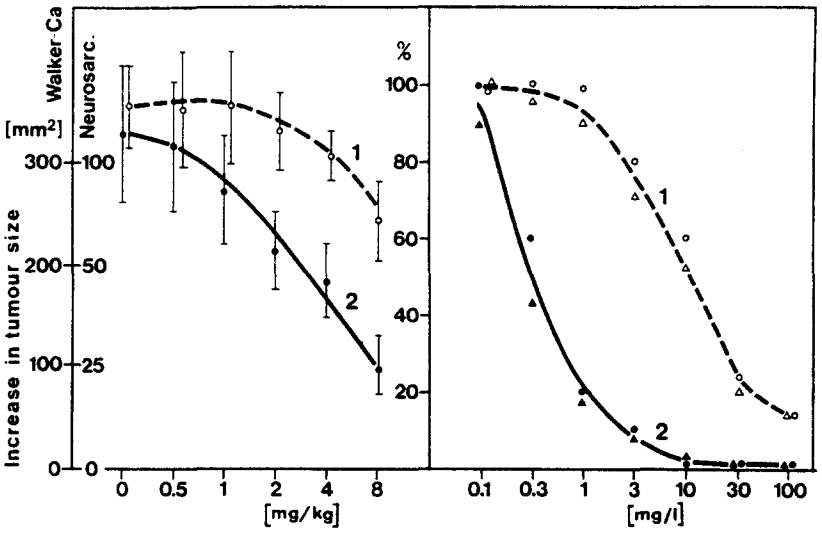

Fig. 1. The effects of different concentrations of adriamycin on the Walker carcinoma or the neurosarcoma in vivo (*left*) *and* in vitro (*right*).

Left: daily increase in tumour size of neurosarcomas (1) *and Walker carcinosarcomas* (2) *with or without adriamycin treatment. Average values* ± *S.D. are from 7 tumours at each point* ($n = 84$ *rats*).

Right: ^{3}*H-Uridine incorporation* in vitro *with adriamycin in tumour cell suspensions from* 2 *tumours each (different symbols) of the neurosarcoma* (1) *and the Walker carcinosarcoma* (2). *Values* (% *of control*) *are the averages from* 2 *cultures with duplicate determinations* (counts/min 5×10^4 *cells*) *at each point.*

cells derived from the neurosarcoma (○ Δ). We have previously obtained similar results with other animal tumours and cytostatic agents [2, 17, 31, 32].

In order to investigate the possibility of using the short-term test to detect acquired resistance to cytostatics in addition to the proliferation-dependent resistance, we developed an adriamycin-resistant tumour cell line from the mouse Sarcoma 180. After treatment with adriamycin over 25 passages, a resistance to this drug was developed which could be observed in animal experiments (Fig. 2, left). The adriamycin-resistance of these tumour cells was also detectable using the short-term test. The non-pretreated (sensitive) tumour cells responded in the *in vitro* test more strongly than the adriamycin-pretreated (resistant) cells. The possibility that the adriamycin-resistant tumour cells had developed cross-resistance to other cytostatics was also investigated using the short-term test. This might be expected with daunomycin, since the two substances are structurally very similar. It was observed that the short-term test did indeed detect cross-resistance to daunomycin in the adriamycin-resistant cells, whereas no resistance was detected against other cytostatic agents (cyclophosphamide, methotrexate) (Fig. 3). The results of these short-term tests were confirmed in animal experiments which demonstrated that adriamycin-resistant tumour cells were indeed insensitive to treatment with daunomycin, but not with other cytostatic agents.

Human tumours

Tests on various human lung and ovarian carcinomas after treatment with adriamycin showed that each tumour exhibited a different dose-response curve (Fig. 4). Some carcinomas were very strongly affected by adriamycin, whereas others showed either moderate effects or almost no response at all. This variable response of the tumours to adriamycin treatment was especially easy to see in the *in vitro* short-term test at an adriamycin concentration of 10^{-2} mg/ml (10 mg/l or 1.7×10^{-5} M). For this reason, the following figures show only the results obtained at this concentration. The results are presented in per cent inhibition. Figure 5 shows the inhibiting effects of adriamycin on ^{3}H-uridine incorporation in tumour cells from various lung and ovarian carcinomas (ordinate) in comparison with control values obtained without adriamycin (abscissa). In general, tumour cells which show high rates of uridine incorporation also show more pronounced inhibitory effects on treatment with adriamycin.

A significant correlation exists between the inhibitory effect of adriamycin on lung tumours and the corresponding effects of daunomycin (D) and 5-fluorouracil (5-FU)

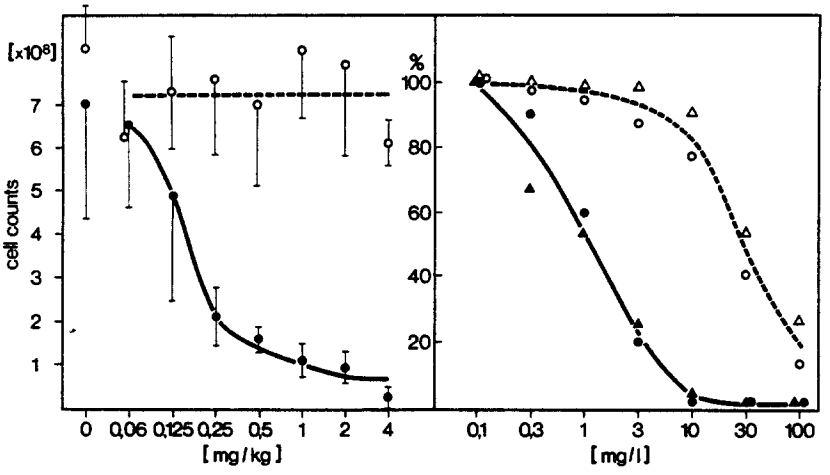

Fig. 2. The effects of different concentrations of adriamycin on the adriamycin-resistant or the adriamycin-sensitive tumour cells of the Sarcoma 180 in vivo (*left*) *and* in vitro (*right*).
Left: adriamycin was injected i.p. on the 3rd and 5th days after transplantation of the tumour cells. The cytostatic effect was measured on the 7th day after transplantation by determination of the cell count using a Coulter counter. ○—○ *adriamycin-resistant;* ●—● *adriamycin-sensitive. Average values ± standard deviations are from 7 tumours at each point (n = 112 mice).*
Right: ^{3}*H-uridine incorporation* in vitro *with adriamycin in adriamycin-resistant* (○, △) *or adriamycin-sensitive* (●, ▲) *tumour cell suspensions from 2 tumours each (different symbols). Values* (% *control*) *are the averages from 2 cultures with duplicate determinations at each point.*

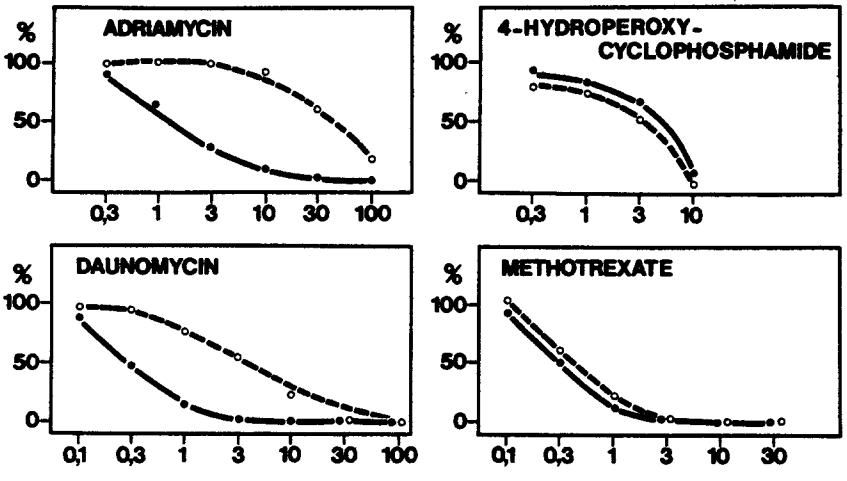

Fig. 3. Dose–response curves (% *of control*) *obtained on application of different cytostatic agents to an adriamycin-resistant* (○—○) *and a sensitive cell line* (●—●) *from the mouse Sarcoma* 180, *as measured with the short-term test.* ^{3}*H-uridine was used for both adriamycin and daunomycin,* ^{3}*H-thymidine and* ^{3}*H-deoxyuridine were used for 4-hydroperoxy-cyclophosphamide and methorexate respectively. Dosage in* μg/ml. *Dosage* 10 *corresponds to* 1.7×10^{-5} M *adriamycin,* 1.9×10^{-5} M *daunomycin,* 3.4×10^{-5} M *4-hydroperoxy-cyclophosphamide and* 2.2×10^{-5} M *methorexate.*

(Fig. 6). The correlation coefficient for the *in vitro* activities of adriamycin and daunomycin is $r = 0.864$ and that for adriamycin and 5-fluorouracil is $r = 0.779$. A similar correlation was also observed between the activities of adriamycin and actinomycin D ($r = 0.907$). It can therefore be seen that cytostatics which have similar structures and mechanisms of action (e.g. adriamycin and daunomycin) exert parallel inhibitory effects *in vitro*. For substances from different structural classes (e.g., adriamycin and 5-fluorouracil) the correlations observed, while still statistically significant, are not as strong. These results seem to

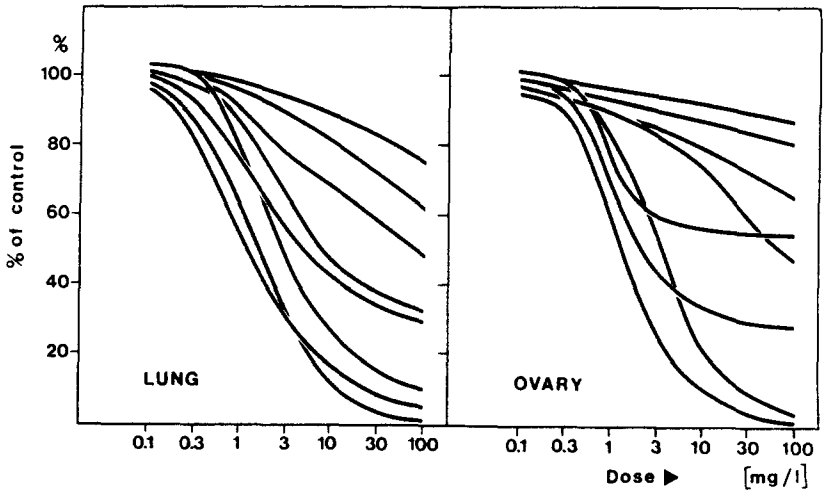

Fig. 4. Incorporation of ^{3}H-uridine in vitro *(% of control) after addition of adriamycin at different concentrations to tumour cell suspensions of individual bronchial and ovarian carcinomas (typical examples are shown).*

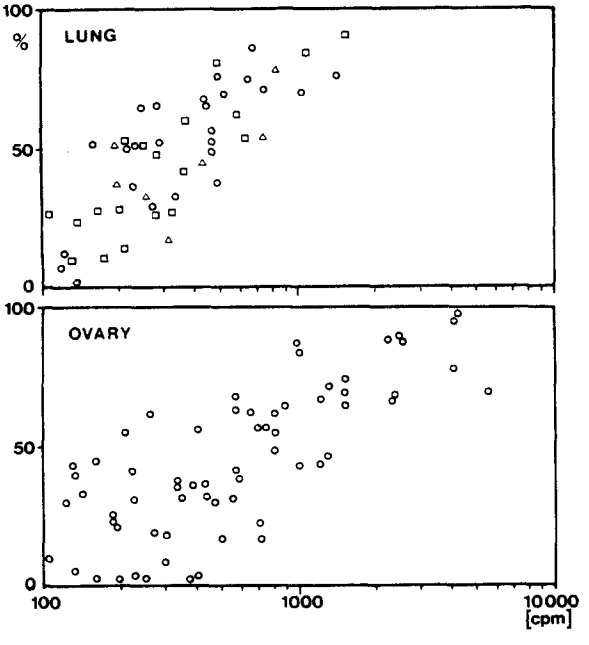

Fig. 5. Relationship between ^{3}H-uridine incorporation in vitro *and the inhibitory activity of adriamycin in the short-term test.*
Abscissa: ^{3}H-uridine incorporation with no cytostatics.
Ordinate: ^{3}H-uridine incorporation after adriamycin treatment (10^{-2} mg/ml; % inhibition).

indicate a differential sensitivity of the tumour cells to individual substances.

In a clinical pilot-study, 40 patients with inoperable lung carcinomas and 10 patients with inoperable ovarian carcinomas were treated by chemotherapy and the success of the treatment compared with the results of the *in vitro* tests (Fig. 7). The patients were distributed into different groups according to the scheme of therapy to be used (A–C: lung tumours, D: ovarian carcinomas). Tumours which responded to clinical therapy are represented in Fig. 7 by the closed symbols and the non-responsive tumours are denoted by the open symbols. Figure 7 clearly shows that tumours which in the *in vitro* test show only a weak reaction to adriamycin treatment (<30% inhibition at a concentration of 10^{-2} mg/ml) also do not respond to chemotherapy in the clinic. Tumours which are strongly inhibited *in vitro* by adriamycin (>30% inhibition) generally show some degree of remission in the clinic after treatment with cytostatics. Only a few false positive test results were obtained. A clear correlation was observed between the inhibitory effects of adriamycin *in vitro* and the clinical response even when a combination therapy without adriamycin was carried out in the clinic (group C). These results indicate that successful clinical therapy can in most cases be predicted from the degree of inhibition induced by adriamycin in the *in vitro* short-term test. Moreover, an unsuccessful therapy can always be detected in advance using this test.

A significant correlation was also observed between the inhibitory effect of adriamycin *in vitro* on the cells from lung and ovarian carcinomas and the corresponding survival rates of the patients (Fig. 8). The larger the inhibition of ^{3}H-uridine incorporation in the tumour cells (ordinate) the longer was the survival time of the corresponding patient (abscissa).

On the basis of the test results using adriamycin, patients with lung and ovarian carcinomas were divided into two groups. One

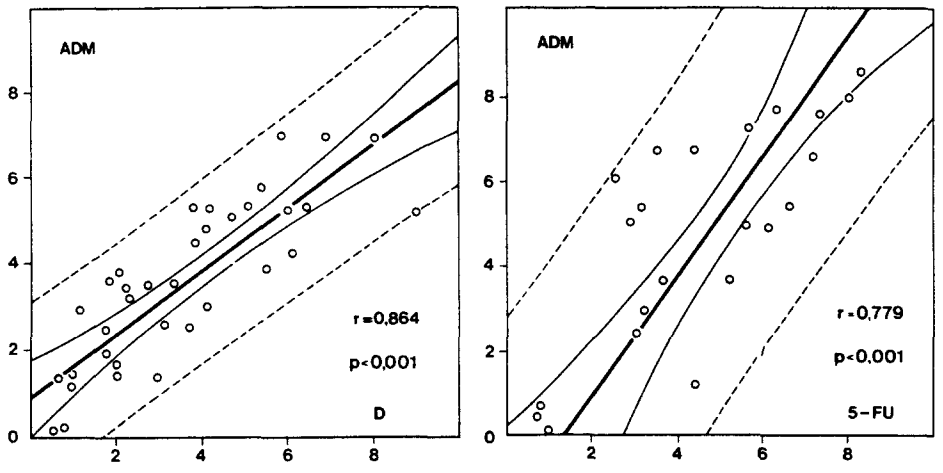

Fig. 6. Correlation between the inhibitory effects (arbitrary units = % inhibition) of adriamycin (ADM) and daunomycin (D) or adriamycin and 5-fluorouracil (5-FU) on ^{3}H-uridine incorporation in vitro. *Regression curves with confidence and tolerance intervals ($\alpha = 0.05$) are also presented.*

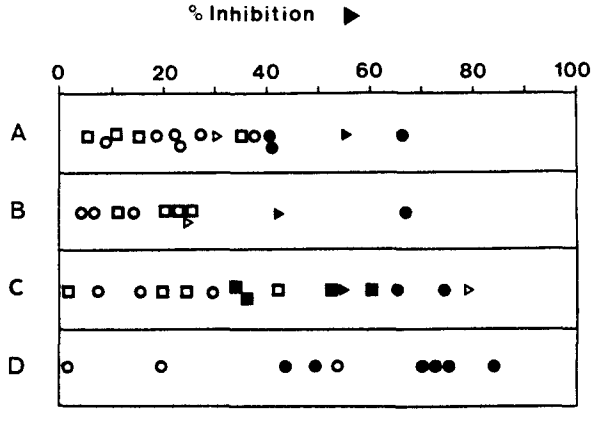

Fig. 7. Comparison of results of in vitro *tests and therapy of inoperable bronchial carcinomas (A–C) and ovarial carcinomas (D). ○ = small cell carcinoma; □ = squamous epithelial carcinoma: ▷ = adenocarcinoma. Values represent the inhibition (%) of uridine incorporation in the tumour cells* in vitro *due to adriamycin* (10^{-2} mg/ml).

Closed symbols: tumours which responded to clinical therapy.

Open symbols: tumours which showed no reaction to chemotherapy.

Schemes of therapy: group A: monotherapy with adriamycin; group B: combination therapy with adriamycin; group C: combination therapy without adriamycin; group D: combination therapy of adriamycin-vincristine. See also Materials and Methods.

group consisted of patients whose tumours had shown only a weak response (<30% inhibition by adriamycin) while the other group contained those patients whose tumours had shown a strong reaction *in vitro* (>30% inhibition by adriamycin). The limit of 30% was obtained from Fig. 7. The survival curves of the patients in the 2 groups are presented in Fig. 9. The survival times of patients with lung carcinomas who were treated by combination therapy without adriamycin are shown on the left side of Fig. 9. It can be seen that patients with tumours shown to be insensitive in the test survived on average 4 months (dashed line), while patients with sensitive tumours survived for 12 months (solid line). The survival rate for untreated patients (dotted line)—the patients had refused chemotherapy—correspond to that of those patients whose tumours proved to be insensitive in the *in vitro* test. In the untreated group of patients, half of the bronchial carcinomas were sensitive when tested *in vitro*. The survival times of patients with ovarian carcinomas who were treated by combination therapy are shown on the right hand side of Fig. 9. Patients with insensitive tumours survived on average for 5 months and those with sensitive tumours for 8 months.

DISCUSSION

The *in vitro* short-term test described above for the pretherapeutic detection of tumour resistance measures the effects of cytostatic agents on the incorporation of radioactive precursors into nucleic acids. This approach has been chosen since most of the cytostatic agents available affect some stage in the metabolism of nucleic acids. Since the rate of nucleic acid synthesis can be taken as a measure of the proliferative activity of a tumour [33], the *in vitro* test can be used to detect proliferation-dependent cytostatic effects [2, 16–21]. It could be demonstrated that the rapidly growing Walker carcinosarcoma and the slowly growing rat neurosarcoma respond differently to chemotherapy with adriamycin. The growth rate of the Walker carcinosarcoma was much more

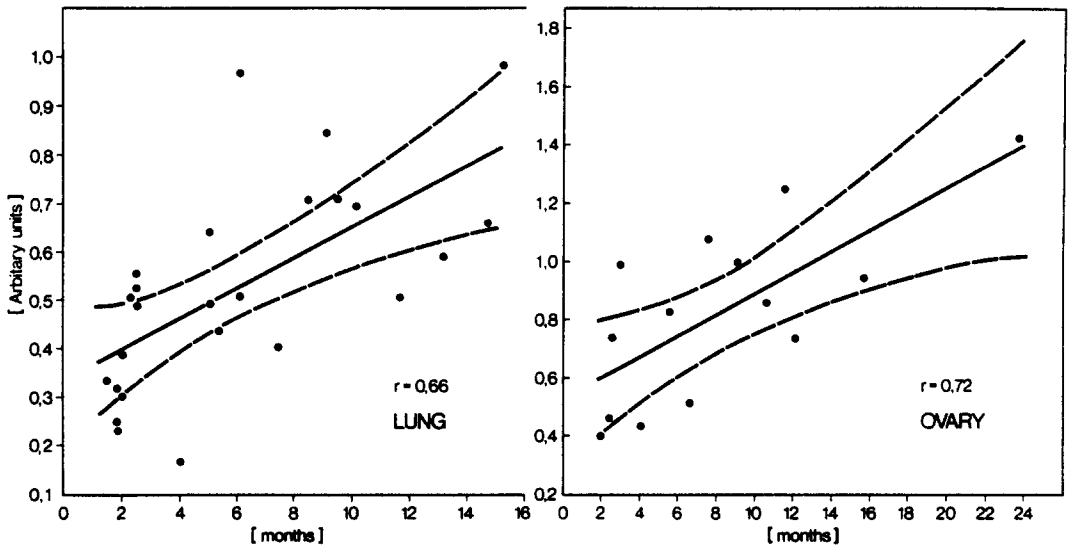

Fig. 8. Correlation between results of tests and survival times of patients with inoperable lung and ovarian carcinomas undergoing therapy (lung: groups A and C; arbitrary units = arc sin % *inhibition).*
Abscissa: survival time (months);
Ordinate: inhibition of ^{3}*H-uridine incorporation due to adriamycin* (10^{-2} mg/ml) in vitro.
Ovary: cyclophosphamide or cyclophosphamide/5-fluorouracil.
From 5 patients with lung carcinoma (2 of group A, 3 of group C) data of the death were not available. Regression curve with confidence intervals ($\alpha = 0.05$).

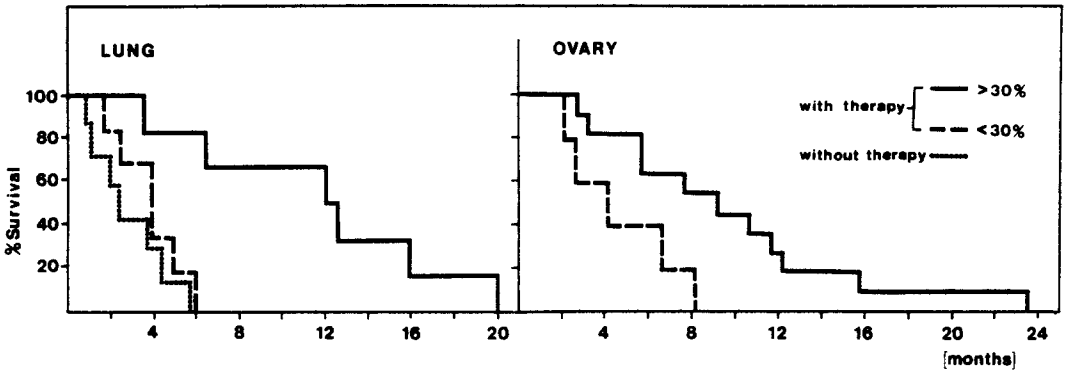

Fig. 9. Survival curves of patients with tumours shown to be resistant (- - - inhibition < 30%, *adriamycin* 10^{-2} mg/ml) *or sensitive (——inhibition* > 30%, *adriamycin* 10^{-2} mg/ml) *in the short-term test.*
Lung: treatment of patients by combination therapy without adriamycin (group C). · · · without therapy.
Ovary: treatment of patients by combination therapy (group D).

strongly reduced by adriamycin than that of the neurosarcoma. This differential sensitivity of the 2 tumours was also observed in the short-term test.

Human bronchial and ovarian carcinomas show individually characteristic reactions to adriamycin *in vitro*. The degree of inhibition attained depends on the rate of nucleic acid synthesis. If a rapidly proliferating tumour responds to adriamycin treatment *in vitro*, in most cases a similar effect can be detected with other cytostatic agents. In addition, tumours which are insensitive to adriamycin are also usually unaffected by other drugs. In this test system, substances which are of the same structural type, as for example, the antibiotics adriamycin and daunomycin, would understandably have biological activities which are more closely correlated than those between drugs from different substance classes. The tumours tested were seen to exhibit individually characteristic sensitivities to the different agents used.

The usefulness of a pretherapeutic test for tumour resistance depends on how well the test results can be correlated with the subsequent course of clinic therapy. From the present investigations it can be concluded that

the results of *in vitro* tests using adriamycin at a concentration of 10^{-2} mg/ml or 1.7×10^{-5} M are largely in agreement with the effects of chemotherapy in the clinic. If tumour cells from lung or ovarian carcinomas are inhibited less than 30% by adriamycin (10^{-2} mg/ml) the same tumours prove to be resistant in the clinic. On the other hand, tumours which are inhibited more than 30% by adriamycin in the short-term test usually respond to clinical treatment. A clear correlation also exists between the inhibitory effects of adriamycin *in vitro* and the growth of the tumour during clinical treatment even when a combination therapy is employed without adriamycin. In a few cases, however, false-positive test results were also obtained. This is hardly surprising, since in the *in vitro* short-term test no account can be taken of various clinical–pharmacological factors such as mode of absorption, metabolism and elimination from the body. When patients with inoperable lung or ovarian carcinomas are distributed into 2 groups on the basis of results of tests with adriamycin—one group showing an inhibition less than 30% and the other greater than 30%—it is observed that the groups have different survival curves. Patients with tumours which were insensitive to the test died sooner than those with tumours which gave an obvious response. Patients with lung cancer who refused chemotherapy lived on average only as long as patients with tumours shown to be resistant *in vitro*. According to our investigations, a test using adriamycin would appear to be sufficient to detect proliferation-dependent tumour resistance. However, it is not satisfactory to take simply the amount of incorporation of radioactive nucleic acid precursors as a measure of the rate of proliferation of the tumour. The absolute value for the incorporation in the cell suspensions can be influenced by various factors, for example varying degrees of damage to the tumour cells during cell isolation or differences in nucleotide pool sizes of individual tumours. In this way tumours which have similar proliferative activities *in vivo* can demonstrate different incorporation rates for radioactive precursors *in vitro*. However, these considerations can, to a large extent, be ignored if the inhibitory effects of adriamycin *in vitro* are presented as percentage values.

Under the present conditions, when no pretherapeutic tests of tumour resistance are carried out, comparative studies on different therapeutic schemes are performed with no distinction being made between sensitive and resistant tumours, which are consequently treated in the same way. The results presented here now offer the possibility of testing the effectiveness of various schemes of therapy using only sensitive tumours.

Another criterion for the suitability of the *in vitro* test for the clinic lies in its ability to detect induced resistance. The short-term test has been used to detect resistance to various cytostatics in some animal transplanted tumours. Using the mouse Sarcoma 180, a resistance to adriamycin was developed *in vivo* [34]. This resistance was detectable both in animal experiments and in the short-term test. Similarly, resistance against cyclophosphamide which was developed in the Walker carcinosarcoma was also found on incubation of the tumour cells *in vitro* with activated cyclophosphamide [35].

In the case of the mouse leukaemia L1210, acquired resistance against cytosine arabinoside and daunomycin could be detected in the short-term test (unpublished results). Human ovarian carcinomas which initially responded to a combination therapy with vincristine and adriamycin in the clinic were also sensitive to adriamycin *in vitro*. If these tumours then showed signs of progression during the further course of clinical therapy, the tumour cells when tested *in vitro* proved to be insensitive to adriamycin (unpublished results). Although tests with adriamycin alone are sufficient to detect proliferation-dependent tumour resistance *in vitro*, the demonstration of induced biochemical resistance requires the use of various cytostatic agents in the short-term test. For each substance to be tested, the most suitable radioactive precursor and the optimal concentration of the substance must be used. Compounds which are rapidly degraded in the organism have much greater activities *in vitro* than *in vivo*. Difficulties are also observed with substances which are only transformed into the activated form within the organism. For this reason, we use a peroxy derivate of cyclophosphamide for *in vitro* tests. 4-Hydroperoxycyclophosphamide, itself inactive, is quite stable at low temperature but is transformed into the cytostatically active form under physiological conditions *in vitro* [32, 36].

Whether or not short-term tests can be applied as a basis for individual tumour therapy is a question which can only be answered by randomised studies, the results of which can be evaluated statistically. At present, the results of studies of this kind are not available. Until such studies are completed

and shown to be positive, the use of *in vitro* tests routinely in the clinic cannot be recommended. In the clinical studies presented above, the normal therapeutic schemes were employed and the test results were retrospectively compared with the degree of success of the treatment.

REFERENCES

1. L. M. VAN PUTTEN, P. LELIEVELD and L. K. J. KRAM-IDSENGA, Cell-cycle specificity and therapeutic effectiveness of cytostatic agents. *Cancer Chemother. Rep.* **56,** 691 (1972).
2. M. VOLM and J. MATTERN, Individuelle Chemotherapie solider Tumoren. *Med. Welt* **27,** 1171 (1976).
3. N. T. BECH-HANSEN, F. SARANGI, D. J. A. SUTHERLAND and V. LING, Rapid essays for evaluating the drug sensitivity of tumor cells. *J. nat. Cancer. Inst.* **59,** 21 (1977).
4. I. J. BICKIS, I. W. D. HENDERSON and J. H. QUASTEL, Biochemical studies of human cancer. *In vitro* estimation of individual tumor sensitivity to anticancer agents. *Cancer (Philad.)* **19,** 103 (1966).
5. U. P. COBB, D. G. WALKER and J. C. WRIGHT, Comparative chemotherapy studies on primary short-term cultures of human normal, benign and malignant tumor tissues—a five year study. *Cancer Res.* **21,** 583 (1961).
6. E. F. KNOCK, R. M. GALT, Y. T. OESTERAND and R. SYLVESTER, *In vitro* estimate of sensitivity of individual human tumors to antitumor agents. *Oncology* **30,** 1 (1974).
7. D. KUMMER, Cytostatica- und Röntgenstrahleneffekte im Nukleinsäurestoffwechsel von soliden malignen Tumoren *in vitro*. *Z. Krebsforsch.* **74,** 76 (1970).
8. H. LIMBURG and M. KRAHE, Die Züchtung von menschlichem Krebsgewebe in der Gewebekultur und seine Sensibilitätstestung gegen neuere Zytostatika. *Dtsch. med. Wschr.* **89,** 1938 (1964).
9. H. J. SEIDEL, Zur Sensibilitätsbestimmung von Tumoren in vitro. II. Testungen am soliden Ehrlich–Carcinom der Maus und an einem menschlichen Colon-Carcinom nach Heterotransplantation (GW 77). *Z. Krebsforsch.* **74,** 131 (1970).
10. ST. TANNEBERGER and G. BAGIGALUPO, Die Benutzung von Zellkulturen zur Ermittlung der Sensibilität menschlicher Tumoren gegenüber Zytostatika. *Dtsch. Gesundh. Wes.* **22,** 11 (1967).
11. M. VOLM, M. KAUFMANN, H. HINDERER and K. GOERTTLER, Schnellmethode zur Sensibilitätstestung maligner Tumoren gegenüber Cytostatika. *Klin. Wschr.* **48,** 374 (1970).
12. W. H. WOLBERG and R. R. BROWN, Autoradiographic studies of *in vitro* incorporation of uridine and thymidine by human tumor tissue. *Cancer Res.* **22,** 1113 (1962).
13. G. P. WÜST and K. J. MATTHES, *In vitro*-Messung des Einbaues von ^{3}H-Thymidin in Jensen-Sarkom unter Cytostatikaeinwirkung mit Hilfe der Flüssigkeits-Szintillations-Spektrometrie. *Z. Krebsforsch.* **73,** 204 (1970).
14. P. P. DENBY (Editor), *Human Tumours in Short Term Culture.* Academic, London, New York, San Francisco (1976).
15. G. P. WÜST (Editor), *Aktuelle Probleme der Therapie maligner Tumoren.* Georg-Thieme, Stuttgart (1973).
16. M. VOLM, J. MATTERN and K. WAYSS, The effects of cytostatic drugs on transplanted tumours. An investigation of the correlation between *in vivo* and *in vitro* results. *Arch. Geschwulstforsch.* **43,** 137 (1974).
17. M. VOLM, M. KAUFMANN, J. MATTERN and K. WAYSS, Sensitivity tests of tumours to cytostatic agents. I. Comparative investigations on transplanted tumours *in vivo* and *in vitro*. *Z. Krebsforsch.* **83,** 85 (1975).
18. J. MATTERN, M. KAUFMANN, H. HINDERER, K. WAYSS and M. VOLM, Sensitivity tests of tumours to cytostatic agents. II. Investigations on human tumours. *Z. Krebsforsch.* **83,** 97 (1975).
19. M. VOLM, M. KAUFMANN, J. MATTERN and K. WAYSS, Möglichkeiten und Grenzen der prätherapeutischen Sensibilitätstestung von Tumoren gegen Zytostatika im Kurzzeittest. *Schweiz. med. Wschr.* **105,** 74 (1975).

20. M. VOLM, M. KAUFMANN, K. WAYSS and J. MATTERN, Resistenztestung von menschlichen Tumoren im Kurzzeittest. *Klin. Wschr.* **53,** 579 (1975).
21. J. MATTERN, M. KAUFMANN, K. WAYSS, M. VOLM, M. KLECKOW, M. MOSTHAGI and I. VOGT-MOYKOPF, Clinical correlates of *in vitro* effect of adriamycin on advanced lung carcinoma. *Klin. Wschr.* **54,** 665 (1976).
22. K. WAYSS, J. MATTERN, J. SCHUHMACHER and M. VOLM, Einbau eines ^{131}Jod-Desoxycytidinmonophosphat-Protamin-Komplexes in die DNS von tumortragenden Ratten nach partieller Synchronisation mit Hydroxyharnstoff. *Nucl.-Med. (Stuttg.)* **14,** 67 (1975).
23. R. M. O'BRYAN, J. K. LUCE, R. W. TALLEY, J. A. GOTTLIEB, L. H. BAKER and G. BONADONNA, Phase II evaluation of adriamycin in human neoplasia. *Cancer (Philad.)* **32,** 1 (1973).
24. H. O. KLEIN, K. L. LENNARTZ, R. GROSS, M. EDER and R. FISCHER, *In vivo-* und *in vitro*-Untersuchungen zur Zellkinetik und Synchronisation menschlicher Tumorzellen. *Dtsch. med. Wschr.* **97,** 1273 (1972).
25. K. KARRER, N. PRIDUN and E. ZWINTZ, Chemotherapeutic studies in bronchogenic carcinoma by the Austrian study group. *Cancer Chemother. Rep.* **4,** 207 (1973).
26. D. VON FOURNIER, F. KUBLI, H. KUTTIG, H. HINDERER and P. DRINGS, Vergleich der Therapieergebnisse mit Radiogold, Kobalt-60-Teletherapie und Chemotherapie bei 330 Ovarialkarzinomen. *Strahlentherapie* **150,** 280 (1975).
27. H. J. SENN, Zur Therapie des fortgeschrittenen, inoperablen Ovarial-Karzinoms. *Ther. Umsch.* **30,** 651 (1973).
28. M. KAUFMANN, F. KUBLI, P. DRINGS, P. BEUTTLER and D. VON FOURNIER, Die Behandlung fortgeschrittener, sekundär-resistenter Ovarial-Carcinome. In *Gynäkologie und Geburtshilfe. Forschunger-Erkenntnisse.* (Edited by H. Husslein) H. Egermann, Wien (1977).
29. K. W. BRUNNER, *Die nichtchirurgische Behandlung des Bronchuskarzinoms.* Huber, Bern, (1973).
30. W. F. JUNGI, A. C. MAYR, P. SMITH, G. A. NAGEL and H. J. SENN, Erste Erfahrungen mit Adriamycin, einem neuen zytostatischen Antibiotikum, in der Kombinationschemotherapie maligner Tumoren. *Schweiz. med. Wschr.* **102,** 1213 (1972).
31. M. VOLM, L. KRIEG, J. MATTERN and K. WAYSS, Effect of synchronisation on chemotherapy of solid transplanted tumours. *Europ. J. Cancer* **13,** 1099 (1977).
32. M. KAUFMANN, J. MATTERN, M. VOLM, H. J. HOHORST, G. PERER and G. VOELCKER, *In vitro* testing of cyclophosphamide on tumours. *Naturwissenschaften* **62,** 446 (1975).
33. K. WAYSS, J. MATTERN, M. VOLM and J. ZIMMERER, Der Einbau von radioaktiv markiertem Thymidin als Maß für die Wachstumsgeschwindigkeit von Tumoren. *Arch. Geschwulstforsch.* **44,** 307 (1974).
34. M. VOLM and C. LINDNER, Detection of induced resistance in short term test. Adriamycin-resistant Sarcoma 180. *Z. Krebsforsch.* **91,** 1 (1978).
35. M. VOLM, J. MATTERN and K. Wayss, Detection of induced tumour-resistance to cyclophosphamide by *in vitro* short-term test. *Arch. Gynäk.* **223,** 249 (1977).
36. G. VOELCKER, U. DRAEGER, G. PETER and H. J. HOHORST, Studien zum Spontanzerfall von 4-Hydroxycyclophosphamid und 4-Hydroperoxycyclophosphamid mit Hilfe der Dünnschichtchromatographie. *Arzneimittel Forsch.* (Drug Res.) **24,** 1172 (1974).