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MODULATION OF 5-FLUOROURACIL METABOLISM BY THYMIDINE

IN VIVO AND *IN VITRO* STUDIES ON RNA-DIRECTED EFFECTS IN RAT LIVER AND HEPATOMA

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Abstract—The effects of thymidine (TdR) co-administration on the cytotoxicity and incorporation of 5-fluorouracil (5-FU) into RNA of various tissues was studied in rats bearing an ascites hepatoma (AH 130). The role of pyrimidine degradation in determining the modulating effects of TdR on the formation of FU-RNA was studied in hepatocytes and AH 130 cells *in vitro*. TdR (500 mg/kg) potentiated the antitumour effect of 5-FU (150 mg/kg) and also increased host toxicity as judged by changes in body weight. TdR given alone did not significantly affect tumour growth and body weight gain. Examination of the effect of TdR on the incorporation of 5-FU into RNA revealed a differential modulation of RNA-directed toxicity in different tissues. Incorporation of 5-FU into RNA in tumour and bone marrow was increased 2- and 4-fold, respectively. In spleen and kidney the incorporation increased by approximately 50%, but the values did not reach statistical significance. In contrast, the incorporation into RNA of liver and intestinal mucosa was decreased to *ca* 35% of the control. TdR at concentrations of 40 μM–40 mM progressively inhibited the degradation of 5-FU and decreased the incorporation of 5-FU into RNA of hepatocytes *in vitro*. In AH 130 cells *in vitro* TdR did not significantly influence the metabolism of 5-FU and the incorporation into RNA. These results demonstrate that the enhanced incorporation of 5-FU into tumour RNA *in vivo* after pretreatment with TdR is related not to local effects on the tumour cells but rather to an increased bioavailability of the drug. Although co-administration of TdR did not selectively enhance the antitumour effect of 5-FU, a differential toxicity in host tissues was indicated by the modulated incorporation of 5-FU into RNA.

Despite widespread clinical use, the precise mechanisms by which 5-fluorouracil (5-FU) exerts its antiproliferative effect still remain unclear. One mode of action of 5-FU involves the conversion of 5-FU to FdUMP, which binds to thymidylate synthetase and inhibits the *de novo* synthesis of dTMP [1]. Thereby the cellular supply of thymidine nucleotides is depleted and DNA synthesis is inhibited. It is also known that 5-FU is converted to FUTP, which then becomes incorporated into RNA [2]. The incorporation into RNA leads to inhibition of the maturation of ribosomal RNA [3, 4]. Furthermore, the incorporation into RNA results in impaired methylation of low molecular weight nuclear RNA [5] and decreased transcription rate of RNA [6], and incorporation into mRNA may result in miscoding during translation [7]. The RNA-directed cytotoxicity of 5-FU should mainly be related to the impaired processing of rRNA. The relative roles of the DNA-directed and the RNA-directed effects of the drug have been shown to vary depending on the biological system being studied and the experimental conditions used. Either or both of these determinants can be of critical importance for the inhibitory actions of 5-FU on tumour proliferation and for the sensitivity of normal tissues to the drug [8].

When thymidine (TdR) is used in combination with 5-FU, a reversal of the cytotoxicity of 5-FU has been found in some cultured tumour cell-lines, suggesting that inhibition of thymidylate synthetase is the major effect of 5-FU in these cells [9–11]. In other experimental tumours, both *in vitro* and *in vivo*, addition of TdR leads to an enhanced cytotoxicity of 5-FU [12–14]. The latter effect has been correlated with an increased incorporation of 5-FU into RNA [13, 15, 16].

Mechanisms by which TdR may enhance the incorporation of 5-FU into RNA include: (a) inhibition by dTTP of the reduction of 5-FUDP to 5-FdUDP by ribonucleotide reductase, which may lead to increased formation of 5-FUTP [12]; and (b) competition by thymine for pyrimidine degrading enzymes, resulting in an increased cellular exposure to 5-FU [12].

The latter effect should be pronounced *in vivo* since the degradation of pyrimidines takes place mainly in the liver [17–19]. An increased plasma half-life of 5-FU has been demonstrated after supplementation with TdR [20–22] and would be expected to result in increased host toxicity as well as increased tumour toxicity, comparable with a higher dose of

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5-FU alone. Thymidine is, however, reported to increase the therapeutic index of 5-FU in some rodent tumour systems, although it has failed to do so in others [12, 15, 23, 24].

In the present work we have studied the effect of TdR on the RNA-directed action of 5-FU by measuring the incorporation of labelled 5-FU into RNA. The main objectives were: (a) to determine whether TdR potentiated the antitumour activity and host toxicity of 5-FU in rats bearing ascites hepatoma AH 130; (b) to investigate whether TdR could differentially modulate the incorporation of 5-FU into RNA of host tissues; (c) to compare the effect of TdR on the formation of FU-RNA and degradation of 5-FU *in vitro* in hepatocytes and AH 130 cells and to determine whether TdR, in the absence of pyrimidine degradation, may act locally on the tumour cells to increase the incorporation of 5-FU into RNA.

Results are presented demonstrating an increased antitumour activity and host toxicity of 5-FU after supplementation with TdR. An enhanced incorporation of 5-FU into tumour RNA *in vivo* was found to be related not to local effects on the tumour but rather to a decreased degradation of 5-FU in the liver. A differential modulation of the formation of FU-RNA was observed in normal host tissues.

MATERIALS AND METHODS

Materials. 5-Fluoro[6-^{3}H]uracil (1.4 Ci/mmole) was purchased from the Radiochemical Centre (Amersham, Bucks., U.K.); 5-fluorouracil (25 mg/ml) was from Hoffmann-La Roche & Co. AG (Basel, Switzerland); Swim's S-77 medium was from Gibco Europe Ltd. (Paisley, Scotland, U.K.) and thymidine was from Sigma Chemical Co. (St. Louis, MO). Other chemicals used were of analytical grade.

Animals. Male Sprague–Dawley rats (Anticimex, Sweden) weighing 180–200 g were used for the experiments. The animals were kept under constant conditions of light and temperature, and had free access to food and water. The ascites hepatoma AH 130 was kindly given to us by AB Leo (Helsingborg, Sweden) and was maintained by weekly i.p. inoculation of 5×10^6 cells.

In vitro *experiments.* AH 130 cells were harvested on day 6 of tumour growth, washed once in Swim's S-77 medium and further diluted with the same medium. Liver cells were isolated by a collagenase perfusion method [25], washed and diluted with Swim's S-77 medium. Liver cells were incubated at a final concentration of 3×10^6 cells/ml and AH 130 cells at a concentration of 5×10^6 cells/ml. The cells were incubated in Swim's S-77 medium at 37° in a total volume of 4 ml. After a 30 min incubation with TdR of concentrations from 0.1 μg/ml to 10 mg/ml (0.4 μM–40 mM), [^{3}H]-5-FU was added (20 μCi in 50 μl Swim's S-77 medium). The lowest thymidine concentration used was within the physiological serum concentration of TdR in the rat, i.e. 0.6–1.3 μM [26]. The final concentration of [^{3}H]-5-FU was 3.6 μM. Incubation was carried out for another 30 min after which the cell suspensions were centrifuged, and cells and medium separately frozen.

In vivo *experiments.* AH 130 cells were transferred i.p. at an inoculation of 5×10^6 cells. The first 24 hr after inoculation were termed day 0, the next 24 hr day 1, and so on. The effect on tumour growth of 5-FU alone and 5-FU in combination with TdR was determined on day 8, after injection of the substances on day 3. 5-FU (150 mg/kg body wt, 1.2 mmole/kg) and/or TdR (500 mg/kg body wt, 2.1 mmole/kg) were injected i.p. Thymidine was administered in 1.7 ml of Ringer glucose solution 30 min before 5-FU. Control animals received Ringer glucose only.

The animals were anaesthetized by ether and killed, and the tumour was collected from the peritoneal cavity. The volume was determined, and the number of tumour cells and non-tumour cells was counted in a haematocytometer. Non-tumour cells were less than 5% of the number of tumour cells. Rats with less than 100×10^6 tumour cells were excluded. This occurred in 20% of the control rats and in 25% of the rats treated with 5-FU.

The effect of TdR on the incorporation of [^{3}H]-5-FU into RNA of different organs was determined on day 6 of tumour growth. Thymidine (500 mg/kg) was injected i.p. in 2 ml 0.9% NaCl. After 30 min the rats received an i.p. injection of 100 μCi [^{3}H]-5-FU (0.36 μmole/kg). Control animals were given 2 ml 0.9% NaCl before the injection of [^{3}H]-5-FU. The animals were anaesthetized and killed 30 min after the injection of 5-FU. Tumour cells were collected from the peritoneal cavity, liver, spleen, kidney, intestinal mucosa and tibial bone marrow, were sampled and frozen.

Tissue analysis. Tissues and isolated cells were analysed for RNA according to the Schmidt–Tannhauser method as modified by Munro and Fleck [27]. Radioactivity was measured in aliquots from the RNA fraction.

Portions from the incubation medium were analysed for total acid-soluble radioactivity and for labelled 5-FU and α-fluoro-β-alanine by chromatography on pre-coated poly(ethylene-imine)-impregnated thin-layer plates. The spots were identified by corresponding unlabelled compounds. F-β-Alanine was in this study assayed as the radioactivity corresponding to β-alanine. The metabolites were eluted from the cellulose with 0.7 M $MgCl_2$/20 mM Tris–HCl, pH 7.4, and radioactivity was measured. Determinations of radioactivity were carried out with Insta-gel scintillation fluid (Packard) in a Packard liquid scintillation counter.

Statistical analysis of the data was performed according to Student's *t*-test.

RESULTS

In vitro *experiments*

The metabolism of 5-FU was different in hepatocytes and hepatoma cells (Table 1). In the hepatocytes the major part of the radioactivity in the medium was in the catabolic product F-β-alanine after a 30 min incubation with [^{3}H]-5-FU. Only 6% of the acid-soluble radioactivity of the medium was in 5-FU, whereas 52% was in F-β-alanine. In contrast, AH 130 cells did not degrade 5-FU to any significant extent, and no detectable radioactivity was found in F-β-alanine.

When hepatocytes were incubated with TdR

Table 1. Metabolism of [^{3}H]-5-FU in hepatocytes and hepatoma cells *in vitro*. Effect of thymidine (0.1 mg/ml)

	Conditions of incubation	Radioactivity (cpm × 10^{-3}/ml) in medium			RNA specific radioactivity (cpm/mg RNA)
		5-FU	F-β-Alanine	Total radioactivity	
Hepatocytes	5-FU	76 ± 12 (100%)	668 ± 38 (100%)	1281 ± 54 (100%)	31,632 ±5923 (100%)
	TdR + 5-FU	828 ± 44† (1085%)	46 ± 3† (7%)	1549 ± 60* (121%)	13,241 ± 412* (42%)
Hepatoma cells	5-FU	840 (100%)	0	1379 (100%)	85,770 (100%)
	TdR + 5-FU	865 (103%)	0	1348 (98%)	81,481 (95%)

Hepatocytes and AH 130 cells were incubated with [^{3}H]-5-FU (3.6 μM, 1.4 Ci/mmole) for 30 min. Thymidine (TdR, 0.1 mg/ml) was added 30 min prior to [^{3}H]-5-FU. The medium was analysed for total radioactivity and for radioactivity of 5-FU and of F-β-alanine. In the cells the specific radioactivity of RNA was determined. In each experiment the incubations were carried out in duplicate. Results are mean ± S.E.M. from three independent experiments with hepatocytes and two experiments with hepatoma cells. Figures in parentheses are % of control value (5-FU only).

* $P < 0.05$.

† $P < 0.001$.

(0.1 mg/ml) 30 min prior to the addition of [^{3}H]-5-FU, the catabolic activity drastically decreased. The radioactivity of F-β-alanine was only 7% of the control, whereas the radioactivity in 5-FU was 1085% of the control (Table 1). The specific radioactivity of RNA in hepatocytes decreased to 42% of the control. The incorporation pattern of [^{3}H]-5-FU into the hepatoma cells was not affected by a prior incubation with 0.1 mg/ml TdR.

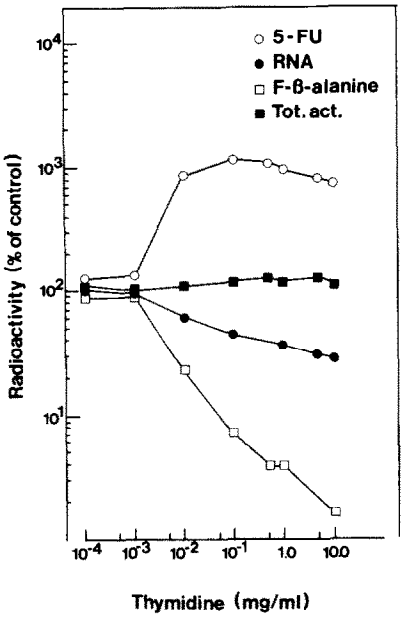

Fig. 1. Effect of thymidine on the metabolism of 5-FU in hepatocytes *in vitro*. Hepatocytes were incubated with [^{3}H]-5-FU (3.6 μM, 1.4 Ci/mmole) for 30 min. Thymidine at increasing concentrations was added 30 min prior to 5-FU. The medium was analysed for total radioactivity and for radioactivity of 5-FU and of F-β-alanine. In the cells the specific radioactivity of RNA was determined. Radioactivity is expressed as % of control values (5-FU only). Absolute values of the control are given in Table 1. In each experiment the incubations were carried out in duplicate. Results are mean from three separate experiments.

To determine the threshold concentration for the effect of TdR, both hepatoma cells and hepatocytes were further incubated with concentrations ranging from 0.1 μg/ml to 10 mg/ml (0.4 μM–40 mM). In the hepatoma cells the radioactivity of RNA in the cells and of 5-FU in the medium was practically unaffected by TdR (not shown). The specific radioactivity of RNA was 93–101% of the control value in the concentration interval 0.1 μg/ml–5 mg/ml TdR. At a TdR concentration of 10 mg/ml, however, there was a slight decrease in the specific radioactivity of RNA to 76% of the control value. No radioactivity was ever demonstrated in F-β-alanine in the hepatoma cell medium.

The incorporation of [^{3}H]-5-FU into hepatocytes was affected by rising concentrations of TdR in the way demonstrated in Fig. 1. Thymidine at a concentration of 0.01 mg/ml had an effect on the incorporation pattern, and with increasing concentrations of TdR the radioactivity of the catabolic product F-β-alanine of the medium steadily decreased, whereas the radioactivity of 5-FU in the medium was higher than the control value. The incorporation of [^{3}H]-5-FU into RNA of the hepatocytes decreased with increasing concentrations of TdR (Fig. 1).

In vivo *experiments*

Tumour growth. The number of tumour cells on day 8 of tumour growth, after various treatments on day 3, is shown in Table 2. In the group treated with TdR only, there was no significant change in the number of tumour cells on day 8. In rats treated with 5-FU, however, there was a significant decrease in tumour cell number to 34% of the control value. Pretreatment with TdR 30 min before 5-FU potentiated the cytotoxic effect of 5-FU and suppressed tumour growth even more effectively. The number of tumour cells on day 8 was only 19% of the control value when the rats were treated with the combination.

Incorporation of [^{3}H]-5-*FU into RNA of different organs* in vivo. The effect of TdR on the incorporation of [^{3}H]-5-FU into RNA of different organs of

Table 2. Effect of tumour growth of 5-FU alone and of 5-FU in combination with thymidine

Treatment on day 3	No. of tumour cells $\times 10^{-6}$	% of control	No. of animals
Control	4134 ± 160	100	6
TdR	3832 ± 258	93	5
5-FU	1411 ± 158*	34	5
TdR + 5-FU	770 ± 166*†	19	7

The number of cells of hepatoma AH 130 was determined on day 8 of tumour growth after various treatments on day 3. Thymidine (TdR, 500 mg/kg) was given i.p. in 1.7 ml Ringer glucose 30 min before 5-FU (150 mg/kg, i.p.). Control rats received Ringer glucose only. Results are mean ± S.E.M.

* Significantly different from control and TdR, $P < 0.001$.

† Significantly different from 5-FU, $P < 0.05$.

tumour-bearing rats is demonstrated in Table 3. There was a significant increase of the specific radioactivity of RNA of tumour and bone marrow to 213 and 396% of the control, respectively, after a prior injection of TdR. In spleen and kidney the incorporation increased by approximately 50%, but the values did not reach statistical significance. In liver and intestinal mucosa the incorporation decreased to 37 and 34%, respectively, of the control.

Body weight. Changes in body weight of tumour-bearing rats after various treatments on day 3 of tumour growth are shown in Fig. 2. Inoculation of tumour cells did not affect the increase in body weight, compared to normal rats, during the first week of tumour growth. Injection of TdR (500 mg/kg) on day 3 of tumour growth did not affect the body weight increase (not shown).

The group treated with 5-FU (150 mg/kg) showed signs of toxicity resulting in a weight loss that was most accentuated on day 7. Thereafter, the rats again increased in weight. An injection of TdR 30 min prior to the treatment with 5-FU potentiated the effect of the drug, and the body weight was significantly lower than after treatment with 5-FU alone ($P < 0.001$).

DISCUSSION

Modulation of the antitumour effect of 5-FU by concurrent administration of TdR has been shown to be related to an increased incorporation of 5-FU into tumour RNA [13, 15, 16]. In our *in vivo* experiments we found a potentiation of the antitumour effect of 5-FU as well as increased incorporation into tumour RNA. *In vitro*, however, TdR did not influence the incorporation of 5-FU into tumour RNA. Accordingly, modulation by TdR of the RNA-directed effects of 5-FU on the tumour cells should be related to a decreased degradation of 5-FU by competition for pyrimidine degrading enzymes in other organs rather than to a local effect on the tumour cells.

We found extensive catabolism of 5-FU in hepatocytes *in vitro*, whereas in AH 130 cells no degradation occurred. This is in agreement with findings by Queener *et al.* [19] showing that tumour cells, with the exception of well-differentiated hepatomas, have a very low catabolic capacity. The liver is considered to be the most important site for pyrimidine catabolism in the body [17–19] and an effect on liver catabolism should therefore influence to a great extent the bio-availability of 5-FU.

Table 3. Effects of thymidine on the incorporation of [^{3}H]-5-FU into RNA of different organs of tumour-bearing rats

Organ	Specific radioactivity of RNA (cpm/mg RNA) NaCl	TdR
Tumour	13,525 ± 636 (100%) (5)	28,850 ± 4081† (213%) (4)
Liver	1832 ± 323 (100%) (5)	686 ± 64* (37%) (4)
Spleen	1414 ± 337 (100%) (4)	2185 ± 271 (155%) (3)
Kidney	1406 ± 269 (100%) (5)	1963 ± 351 (140%) (4)
Intestinal mucosa	3407 ± 742 (100%) (5)	1158 ± 160* (34%) (4)
Bone marrow	439 ± 29 (100%) (5)	1739 ± 172‡ (396%) (4)

On day 6 of tumour growth the animals were given an i.p. injection of TdR (500 mg/kg) or NaCl (0.9%), and 30 min later they received [^{3}H]-5-FU (100 μCi, 0.36 μmole/kg). The animals were killed 30 min after the administration of 5-FU. The specific radioactivity of RNA in different organs was determined. Results are mean ± S.E.M. of duplicate samples from three to five animals, shown in parentheses.

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.001$.

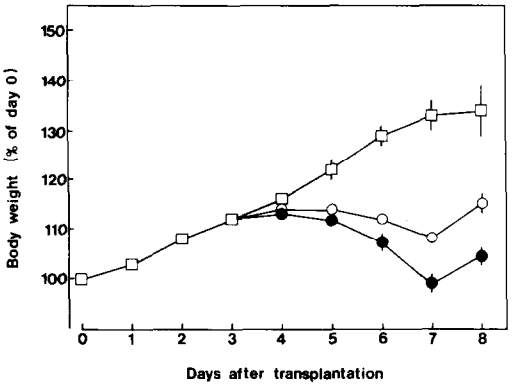

Fig. 2. Effect on body weight of 5-FU alone and 5-FU in combination with TdR. Body weight of tumour-bearing rats (□), (4); tumour-bearing rats treated with 5-FU (150 mg/kg) on day 3 (○), (9); tumour-bearing rats treated with TdR (500 mg/kg) and 5-FU (150 mg/kg) on day 3 (●), (12). Results are mean ± S.E.M. with the number of animals indicated in parentheses. Error bars are only given if they are greater than the symbols.

Addition of TdR to the medium at concentrations above 4 μM resulted in considerable inhibition of 5-FU degradation in hepatocytes, whereas no effect on the metabolism of 5-FU was found in tumour cells. As a consequence *in vivo* of an inhibited degradation of 5-FU by TdR, one would expect an increased bio-availability of 5-FU. In fact, TdR has been shown to increase plasma half-life of 5-FU [20–22] and reduce the amounts of catabolic products in serum [28].

An increased availability of 5-FU and prolonged cellular exposure to the drug is considered to be the main mechanism for the observed increase in potency and incorporation into tumour RNA *in vivo* [29]. However, in some experimental cell systems *in vitro*, a potentiation of the incorporation of 5-FU into RNA has been observed after addition of TdR [14, 30]. The biochemical basis for this effect might be mediated by the inhibition by dTTP of ribonucleotide reductase reduction of 5-FUDP to 5-FdUDP [12] thus promoting the formation of 5-FUTP. Such a mechanism does not seem to operate in AH 130 cells, since no effect of TdR on the incorporation of 5-FU into RNA was observed *in vitro*. The decreased incorporation of 5-FU into RNA of hepatocytes after addition of TdR was probably related to overloading of the pyrimidine degrading enzymes. A decreased degradation of endogenous uracil may lead to expansion of uracil nucleotide pools, and consequently 5-FU has to compete with more endogenous precursors for incorporation into RNA.

The combination with TdR markedly increased the effects of 5-FU *in vivo*. A potentiation of the incorporation of 5-FU into tumour RNA was observed as well as a reduction in tumour growth. Host toxicity was also increased, as judged by the body weight decrease. Moreover, the increased incorporation of 5-FU into bone marrow RNA indicated an increased toxicity in this organ. On the other hand, the incorporation into RNA of intestinal mucosa was decreased.

Whether modulation of RNA-directed effects of 5-FU is the sole determinant of the altered cytotoxicity obtained by pretreatment with TdR is not known. Inhibition of DNA synthesis through 5-FdUMP inhibition of thymidylate synthetase is undoubtedly an important mechanism for the toxicity of 5-FU. Although by addition of TdR the cells may overcome this block [24], the alteration of 5-FU pharmacokinetics may affect the DNA-directed effects when TdR has been cleared from the circulation. Moreover, through the action of thymidine phosphorylase, deoxyribose-1-P released from TdR can be utilized in the conversion of 5-FU to 5-FUdR [22]. Increased levels of circulating 5-FUdR have been observed in patients given 5-FU in combination with TdR [21, 22]. Considering that 5-FUdR is more cytotoxic *in vitro* than 5-FU [31], an increased conversion of 5-FU to 5-FUdR may have profound therapeutic and toxicological consequences. The resultant toxicity of 5-FU in different tissues after modulation by TdR should thus be dependent on the relative importance of RNA- and DNA-directed actions of 5-FU in the different tissues, and would be expected to differ between tissues and species according to the relevant enzyme activities.

Thymidine is reported to increase the therapeutic index of 5-FU in some rodent tumour systems but has failed to do so in a number of others [12, 15, 23, 24]. Clinical trials with 5-FU and TdR in combination have not appeared to improve the therapeutic index of 5-FU [29]. Although antitumour activity is potentiated, marrow toxicity and neurotoxicity are concomitantly increased [20, 32]. In contrast to the increased marrow toxicity, gastrointestinal toxicity is reported as not being increased [29, 32].

The fact that the intestinal toxicity of 5-FU is not potentiated by TdR may possibly be related to a decreased incorporation of 5-FU into RNA as found in our studies on rat intestinal mucosa. Based on the concept of the importance of RNA-directed effects for the cytotoxicity of 5-FU, this may provide a basis for an improved therapeutic index of 5-FU by development of multi-agent treatment protocols.

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REFERENCES

1. K. U. Hartmann and C. Heidelberger, *J. biol. Chem.* **236**, 3006 (1961).
2. N. K. Chaudhuri, B. J. Montag and C. Heidelberger, *Cancer Res.* **18**, 318 (1958).
3. U. Stenram and R. Willén, *Chem.-biol. Interact.* **2**, 79 (1970).
4. D. S. Wilkinson, T. D. Tlsty and R. J. Hanas, *Cancer Res.* **35**, 3014 (1975).
5. R. I. Glazer and K. D. Hartman, *Molec. Pharmac.* **17**, 245 (1980).
6. G. Eriksson and U. Stenram, *Analytical Chemistry Symposia Series*, Vol. 7. Elsevier, Amsterdam (1983).
7. C. E. Myers, *Pharmac. Rev.* **33**, 1 (1981).
8. H. G. Mandel, *Cancer Treat. Rep.* **65** (Suppl. 3), 63 (1981).

9. H. Madoc-Jones and W. R. Bruce, *Cancer Res.* **28**, 1976 (1968).
10. M. Umeda and C. Heidelberger, *Cancer Res.* **28**, 2529 (1968).
11. W. J. Reeves, Jr. and R. Cailleau, *Proc. Soc. exp. Biol. Med.* **131**, 1068 (1969).
12. G. Santelli and F. Valeriote, *J. natn. Cancer Inst.* **61**, 843 (1978).
13. S. Spiegelman, R. Nayak, R. Sawyer, R. Stolfi and D. Martin, *Cancer* **45**, 1129 (1980).
14. J. Fried, A. G. Perez, J. M. Doblin and B. D. Clarkson, *Cancer Res.* **41**, 2627 (1981).
15. S. Spiegelman, R. Sawyer, R. Nayak, E. Ritzi, R. Stolfi and D. Martin, *Proc. natn. Acad. Sci. U.S.A.* **77**, 4966 (1980).
16. D. S. Martin, R. Nayak, R. C. Sawyer, R. L. Stolfi, C. W. Young, T. Woodcock and S. Spiegelman, *Cancer Bull.* **30**, 219 (1978).
17. N. K. Chaudhuri, K. L. Mukherjee and C. Heidelberger, *Biochem. Pharmac.* **1**, 328 (1958).
18. P. Fritzson, *J. biol. Chem.* **237**, 150 (1962).
19. S. F. Queener, H. P. Morris and G. Weber, *Cancer Res.* **31**, 1004 (1971).
20. J. M. Kirkwood, W. Ensminger, A. Rosowsky, N. Papathanasopoulos and E. Frei, *Cancer Res.* **40**, 107 (1980).
21. T. M. Woodcock, D. S. Martin, L. A. M. Damin, N. E. Kemeny and C. W. Young, *Cancer* **45**, 1135 (1980).
22. J.L-S. Au, Y. M. Rustum, E. J. Ledesma, A. Mittelman and P. J. Creaven, *Cancer Res.* **42**, 2930 (1982).
23. M. W. Trader, F. M. Schabel, W. R. Laster, M. H. Witt and T. H. Corbett, *Proc. Am. Ass. Cancer Res. ASCO* **20**, 95 (1979).
24. D. S. Martin, R. L. Stolfi, R. C. Sawyer, R. Nayak, S. Spiegelman, C. W. Young and T. M. Woodcock, *Cancer* **45**, 1117 (1980).
25. P. O. Seglen, in *Methods in Cell Biology* (Ed. D. M. Prescott), Vol. XIII, p. 29. Academic Press, London (1976).
26. H. Nottebrock and R. Then, *Biochem. Pharmac.* **26**, 2175 (1977).
27. H. N. Munro and A. Fleck, *Analyst* **91**, 78 (1966).
28. T. T. Lee, Y. M. Rustum and S. Bjornsson, *Proc. Am. Ass. Cancer Res.* **20**, 123 (1979).
29. C. W. Young, T. M. Woodcock and D. S. Martin, *Cancer Treat. Rep.* **65** (Suppl. 3), 83 (1981).
30. J. Maybaum, B. Ullman, H. G. Mandel, J. L. Day and W. Sadee, *Cancer Res.* **40**, 4209 (1980).
31. B. Ardalan and R. Glazer, *Cancer Treat. Rev.* **8**, 157 (1981).
32. S. J. Vogel, C. A. Presant, G. A. Ratkin and C. Klahr, *Cancer Treat. Rep.* **63**, 1 (1979).