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Apoptosis and cancer chemotherapy

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SUMMARY

The major disseminated cancers remain stubbornly resistant to systemic therapy. Drug-resistant tumours include both slow and fast growing types, with the carcinomas constituting the major problem. Strategies for drug discovery have, in the past, been focused on attempts to design antiproliferative agents, largely targeted to interfere with DNA integrity and replication. The malignant phenotype might be characterized by the emergence of cell populations with a greater survival potential: a lower proclivity to undergo apoptosis. This idea provides a possible explanation of the genesis and progression of cancer and of the inherent resistance of tumour cells to engage apoptosis. Work is described which identifies the molecular basis for differences in the survival potential of stem cells in the crypts of the colon and small intestine. The advantageous survival of colonic stem cells, provided by expression of bcl-2 and a muted p53 response to DNA damage, allows damaged cells to survive. Continued expression of bcl-2 renders tumour cells resistant to drug-induced DNA damage by a mechanism different from classical mechanisms of drug resistance. The attenuation of cell survival is described as a key component in strategies for the drug treatment of disseminated cancers.

1. INTRODUCTION

Cancer pharmacologists continue to struggle with the quest to discover cellular targets at which innovative drug molecules might be aimed. The past four decades have seen only modest success in this endeavour, with the major human cancers continuing to reap significant numbers of lives. The vast increase in knowledge regarding the molecular basis of malignancy, facilitated by advances in molecular biology, has provided a plethora of new potential targets for the aspiring drug discoverer. However, few of these afford an immediately obvious and selective means of preventing tumour growth without affecting that of normal tissues. Moreover, the work of Foulds (1958) has long alerted us to the fact that not one but a series of genetic changes are likely to have occurred prior to the genesis of a frankly malignant cell; on which one of these multiple events should the drug discoverer focus attention? Is there a hierarchy among these events, so that a single 'magic bullet' could be aimed at a critical biochemical feature underpinning the malignant phenotype? And if that critical target were to be modulated by a drug, what should we expect as the end-point of therapy: restoration of normal cellular function, cytostasis of the malignant cell, or its death? It is in asking this latter question that a profound change in perception of the problems of chemotherapy has recently taken place, a change consequent on our understanding that targeting a drug to a key feature of cellular biochemistry is only a first step, with subsequent steps and the final outcome being determined by a cellular response to a drug-induced change in homeostasis (Dive & Hickman 1991). Observations that many types of current anticancer drugs, with completely disparate cellular targets, induced apoptosis in susceptible cells (reviewed by Hickman 1992; Dive & Wyllie 1993) played a key feature in this thinking, and suggested that disruption of homeostasis and/or cellular damage initiated a cellular suicide response, or not, according to phenotype. Thus, the end-point of drug therapy was dependent on a cellular response and not solely on the quality or quantity of change induced by the drug. Here lay a possible explanation of what has been termed 'inherent' drug resistance: those tumours that have responded well to therapy may be derived from cells whose response to damage was to engage apoptosis readily; those which were resistant either do not receive the stimulus for cell death (classical mechanisms of drug resistance) or, more importantly, do not respond to it. Our failure to affect many of the solid tumours may reflect the high survival potential of their cells of origin in comparison to tissues like the bone marrow. Evidence to support this idea is described here.

The implications stemming from the idea that the end point of the action of drugs may be dependent upon a cellular response (simple stimulus–response coupling, figure 1) are considerable. First, as stated

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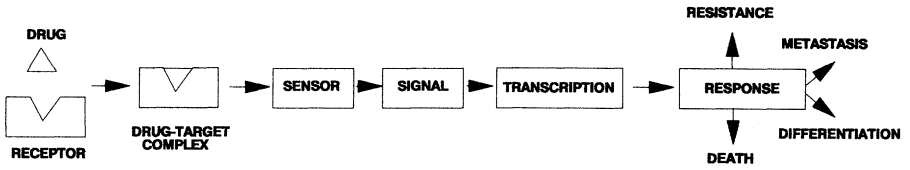

Figure 1. Stimulus response coupling. Drug-induced perturbations of cellular metabolism are presumably 'sensed' by the cell and signals initiated to engage the appropriate response, according to phenotype. One of these responses is to engage apoptosis. It is suggested that at the 'coupling' stage there is modulation of the signal. The outcome of drug treatment is therefore dependent upon the nature of these modulatory events.

above, the failure of drugs to impact on many of the major tumours may be a reflection of the failure of the tumour cells to undergo apoptosis at a threshold of cellular perturbation below that triggering the death of cells in normal tissue, such as the bone marrow or the epithelia of the small intestine. Essentially, this idea suggests that these refractive tumours are resistant to the process of engaging cell death, not to the drugs per se. Secondly, the conundrum of choosing a target from among the many progressive changes in cellular biochemistry characterizing the progression to malignancy may be resolved: selective perturbation of *any* of these changes may be imposed as long as the cell is able to couple this perturbation to the engagement of apoptosis. Is this realizable?

Critical to making an advance is the identification of those factors which modulate the cellular response to damage: the cellular threshold defining whether to survive or to die. The goal of inhibition of cellular survival has the added advantage that it is not necessarily dependent on cell proliferation biochemistry, an advantage important to the therapy of the many slow growing tumours with a low growth fraction.

We, like others, have tested the idea that modulation of the coupling of the response to damage may play a role in drug resistance (Fisher *et al.* 1993; Merritt *et al.* 1994*a*). The therapy of colon carcinoma was of particular interest for two reasons. First, because it is a tumour for which chemotherapy is only palliative. Secondly, because a study of the tissue from which it originates provided insight to the role that modulation of apoptosis may provide in carcinogenesis and the survival of damaged cells which appear to be able to withstand the further damage imposed by chemotherapeutic drugs and radiation.

2. COLON CARCINOGENESIS: INSUFFICIENT ALTRUISTIC APOPTOSIS

U.K. statistics, from the Cancer Research Campaign, estimated that 28 590 cancers arose in the colon in 1987, accounting for a tenth of deaths from cancer in 1991. A puzzle of bowel cancer has been the observation that less than 5% of cancers arise in the small intestine, with the majority in the colon and rectum (Goligher 1980). Elegant studies comparing the kinetics of cell proliferation in small intestine and colon, both in the rodent and in man, have not revealed differences which may account for the greater incidence of colon cancer (reviewed by Potten 1992). Rather, morphological studies have revealed that patterns of cell loss by apoptosis from the small intestine and colon are quite distinctive and provide a possible explanation of the differential cancer incidence (Potten *et al.* 1992). Our investigations suggest that the survival of damaged stem cells in the colon may allow the expansion of a population with a greater survival potential than the small intestine, and that maintenance and/or an increase of this survival potential may render the tumour inherently chemoresistant.

(*a*) *Stem cell apoptosis is restricted to the small intestine*

Careful analysis of cell positional behaviour and hierarchies in the crypts of the murine colon and small intestine by Potten and colleagues (reviewed in Potten 1992) suggested that the topology of cell renewal in colonic and small intestinal crypts was quite different (figure 2). In the small intestine, the putative stem cells were assignable to cell positions 4 or 5 (figure 2) whereas in the colon, stem cells were positioned at the very base of the colonic crypt, from positions 1 to 2. As in all dynamic tissues, homeostasis in the intestinal crypts appears to be maintained by removing cells excess to requirement by apoptosis. Most interestingly, the pattern of this spontaneous apoptosis was also different in the small intestine and colonic crypts: in the small intestine, apoptotic cells were observed to be restricted to the stem cell region whereas in colonic crypts spontaneous apoptosis was less frequent and occurred in a less topologically restricted way (reviewed by Potten 1992). Critically, few apoptotic cells were observed at the base of colonic crypts, harbouring the stem cells. This differential pattern of apoptosis was exaggerated when animals were exposed to DNA damaging agents or radiation (Ijiri & Potten 1987).

These observations of the differential amounts and position of apoptosis in the crypts led to the hypothesis that damaged small intestinal stem cells were deleted by an 'altruistic' apoptosis whereas in the colon, damaged cells survive and may go on to give rise to cancers (Potten *et al.* 1992). Perhaps the most critical step in carcinogenesis is that a cell capable of replication is able to survive a transforming mutation and to progress through further genetic changes because of a continued, enhanced ability to sustain

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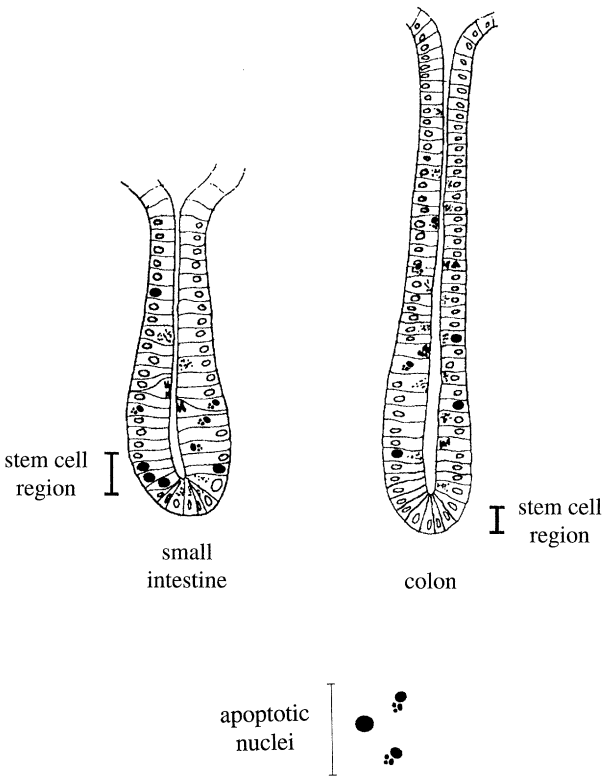

Figure 2. Cartoon of the appearance of longitudinal sections of the crypts from the murine small intestine and colon. The stem cell regions are shown together with the positions of cells which are observed to undergo spontaneous or induced apoptosis. The stem cell in the small intestine is considered to be at position 4 up from the bottom, above the Paneth cells, and at position 1 in the colon, at the very base of the crypt.

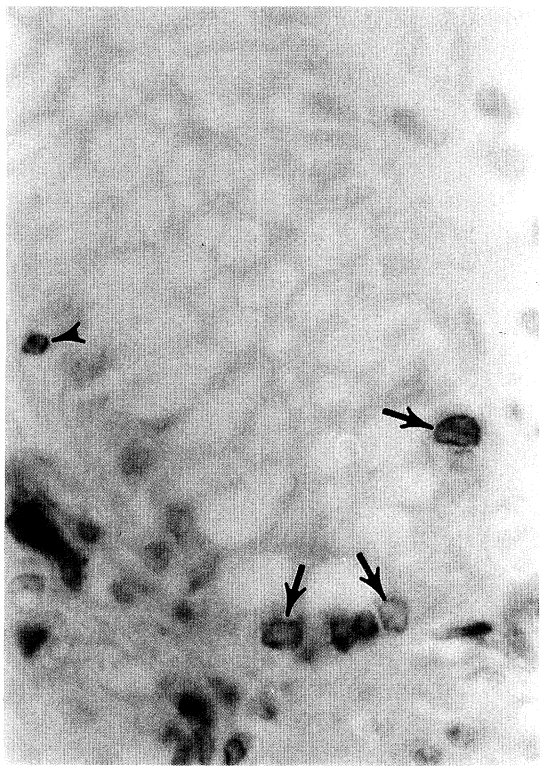

Figure 3. Immunohistochemical analysis of the expression of the human bcl-2 protein, by immunoperoxidase staining, in a normal colonic crypt.

and survive damage. This damage may include that imposed by radiation therapy or chemotherapeutic drugs. What is the genetic basis of this survival advantage and how might it relate to chemoresistance?

(*b*) Bcl-*2 is differentially expressed between the small intestine and colon*

The idea that the expression of certain genes could modulate survival was made a reality by the discovery that the activity of the gene *bcl*-2 was to inhibit apoptosis (reviewed by Reed 1994). Using established methods to prepare longitudinal sections of mouse and human colonic and small intestinal crypts, the expression of *bcl*-2 was investigated by us using immunohistochemistry (Merritt *et al.*, unpublished data). We found that expression of bcl-2 was confined to the colonic epithelia, with little staining in the small intestine. Peroxidase staining of sections from normal human colonic crypts suggested strong perinuclear staining in epithelial cells at the base of the crypt, precisely among the stem cell population (figure 3). The sporadic staining of the epithelial cells of the small intestine was not confined to the stem cell region, and may have been of intraepithelial lymphocytes. Thus *bcl*-2 expression was in those cells which resist either spontaneous or induced apoptosis. The result contrasted with a pattern of staining reported by Hockenbery *et al.* (1991) who claimed that the gene was expressed equally in the crypts of the small intestine and colon. Their data are difficult to reconcile with observations of topographically restricted patterns of both spontaneous and induced apoptosis (see figure 2). The selective expression of *bcl*-2 presumably protects epithelial stem cells from the colon from excessive loss induced by concentrating toxins from the diet or from the colonic flora.

(*c*) *Colon adenocarcinomas express* bcl-*2*

In a limited but on-going survey of both human and murine colonic tumours, we found significant expression of *bcl*-2. Interestingly, immunofluorescence staining showed bcl-2 protein to be largely perinuclear in distribution, with occasional intranuclear expression (Merritt *et al.*, unpublished data). Very significant staining of bcl-2 protein has been reported recently in other solid tumours: in carcinomas of the breast (Leek *et al.* 1994), small cell lung cancer (Pezzella *et al.* 1993; Ikegaki *et al.* 1994), androgen-independent prostate cancer (McDonnell *et al.* 1992; Colombel *et al.* 1993) and in neuroblastomas (Castle *et al.* 1994). In the studies of breast carcinomas (Leek *et al.* 1994), it appeared that the loss of bcl-2 expression in the tumours with a poorer prognosis was associated with the emergence of positive immunohistochemical staining for p53, c-erbB-2 and EGFR (epidermal growth factor receptor). This may be interpreted as

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representing progression to a higher survival threshold than that provided by bcl-2 alone, and that in this process of tumour progression, the expression of bcl-2, initially required for enhanced survival, may ultimately be lost. Interestingly, in the breast carcinomas there was an inverse relationship between the expression of p53 and bcl-2 (Leek *et al.* 1994). As described below, this also appears to hold for the crypt epithelia of the gut.

(*d*) *Expression of* bcl-*2 prevents cell death induced by drugs used to treat colon cancer*

The model of stimulus–response coupling discussed above (figure 1) predicts that the expression of genes which modulate apoptosis, like bcl-2, should modulate the response of cells to drug-induced damage. As a mechanism of drug resistance, this is novel. Previous paradigms for drug resistance have included only changes to what is here called the 'stimulus': the amount and quality of damage delivered. The cell has been perceived to be active only in modulating the amount of this stimulus, either by inactivating the drug or effluxing it, by changing the 'target' (for example by utilizing alternative biochemical pathways, or by quantitative or qualitative changes in the target) or by repairing drug-induced damage, for example to DNA.

We chose to study whether the ectopic expression of *bcl*-2 could provide resistance to a drug class without changing the 'stimulus' in any way. It was therefore essential that we could define and quantitate the stimulus. For this reason we investigated inhibitors of the enzyme thymidylate synthase. There are very specific inhibitors of this enzyme, belonging to different chemical classes of agent with different transport routes across the cell membrane. In addition, the amount of enzyme and its activity can readily be measured, and the results of its inhibition can be quantitated (by changes in pools of thymidine and other nucleotides). After treatment of cells with inhibitors of thymidylate synthase, reduction of pools of thymidine result in a gradual incorporation of deoxyuridine into nascent DNA. This leads to an accumulation of strand breaks, which again can be quantified. Indeed, it has generally been supposed that these breaks are the lethal event induced by this class of drug: DNA breaks in some critical, but as yet unidentified gene(s) proving to be lethal to the cell. With respect to our studies of cell death and carcinogenesis in the colon, these drugs, and particularly 5-flourouracil, are the major class of chemotherapeutic agents administered to patients with colon cancer and we were keen to discover what effect the expression of *bcl*-2 might have on drug sensitivity.

As an easily manipulated model system we used human lymphoma cells transfected with human *bcl*-2 or a vector control (Fisher *et al.* 1993). The transfected cells were found to contain about three times the amount of bcl-2α protein found, by Western blotting, in a naturally *bcl*-2 expressing lymphoma. Both the transfects divided at the same rate and had an equal cell cycle time (24 h) and distribution, important when comparing S-phase-specific drugs. In a detailed study of the effects of 5-fluorodeoxyuridine, it was shown that transfection of *bcl*-2 did not alter the

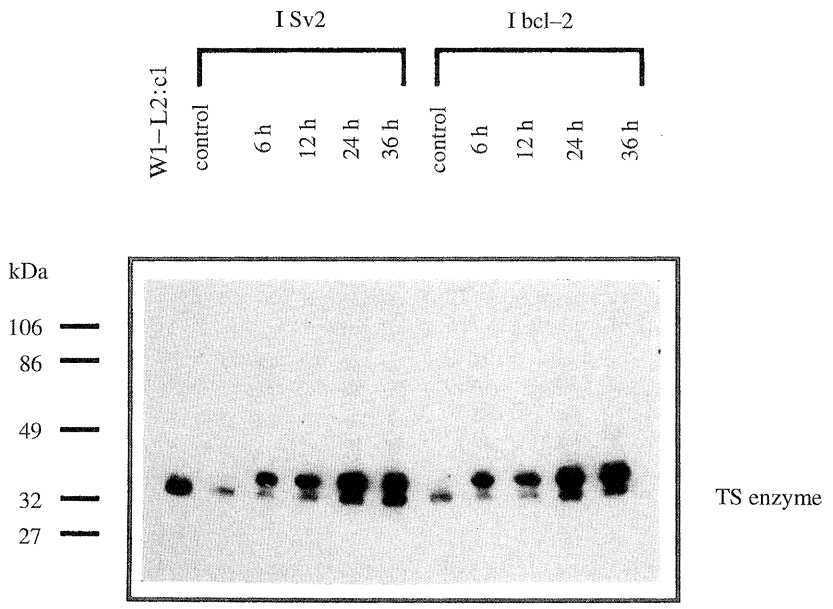

Figure 4. Western blot of the thmidylate synthase enzyme in human B-cell lymphoma cells transfected with either vector (I Sv2) or human bcl-2 (I bcl-2). The blot shows changes in the amount of enzyme after various times of incubation of the cells with 5-fluorodeoxyuridine, as the cells attempt to overcome the blockade of the enzyme. Also, with time, the blot shows the appearance of the higher molecular mass form of thymidylate synthase formed as the drug forms a ternary complex. (The lane WI-L2:c1 contains purified thymidylate synthase protein.) (From Fisher *et al.* (1993), used with permission.)

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amount of drug binding, via the formation of a ternary complex to the thymidylate synthase enzyme, as detected by Western blotting, nor was the inhibition-stimulated up-regulation of the enzyme inhibited (figure 4). Analysis, by alkaline elution, of DNA damage in nascent DNA showed that, with time, strand breaks occurred in both the vector and bcl-2 transfected cells (figure 5). Twelve hours after removal of 1 μM of the drug the cells had equivalent amounts of DNA damage, and this continued to increase, with the bcl-2 transfectants apparently accumulating more damage (figure 5). This amount of DNA damage would normally be expected to be lethal to both vector transfects and bcl-2 transfects. In fact, whereas cells transfected with the vector alone readily underwent apoptosis after a 24 h treatment with 5-fluorodeoxyuridine, the bcl-2 expressing cells remained viable (figure 5). Similar results were observed using a series of quinazoline-based thymidylate synthase inhibitors which differed with respect to their membrane transport (passive or facilitated) and their intracellular metabolism: these compounds either were or were not capable of undergoing polyglutamation. Overall, our data clearly showed that *bcl*-2 expression prevented cell death independently of the classical mechanisms of drug resistance associated with this class of agent.

The survival of cells with accumulating DNA damage (figure 5) is remarkable. It remains to establish the fate of these cells and what the integrity of their DNA might be at later times. Since drug-treated bcl-2 transfectants repopulated the cultures (Fisher *et al.* 1993) we presume that DNA was repaired. Whether this repair was with high fidelity, or not, is a very important question: inhibition of thymidylate synthase has previously been shown to increase homologous recombination activity, and it could be presumed that such an event, in cells surviving with high levels of DNA damage, might promote the development of further mechanisms of resistance.

Bcl-2 has now been reported to inhibit cell death induced by a whole variety of chemotherapeutic drugs with different loci of action. This includes glucocorticoids, methotrexate, etoposide (Miyashita & Reed 1993), nitrogen mustard and camptothecin (Walton *et al.* 1993) and X-irradiation (Collins *et al.* 1992). This truly pleotropic resistance extends far beyond the range of drugs to which the so-called multidrug resistance phenotype applies (reviewed by Twentyman 1992). Importantly, in etoposide-treated CH31 murine B cells transfected with *bcl*-2 there was shown to be an increase in the clonogenic potential of the resistant *bcl*-2 containing cells, although like our results with fluorodeoxyuridine (above) no change was observed in the amount of DNA damage induced nor, critically, in the rate of repair (Kamesaki *et al.* 1993).

These experiments support the idea that the sensitivity of tumour cells to drugs is not solely dependent on the type or quantity of perturbation delivered by the drugs: the consequences of this for the designer of new drugs are considerable, as outlined below.

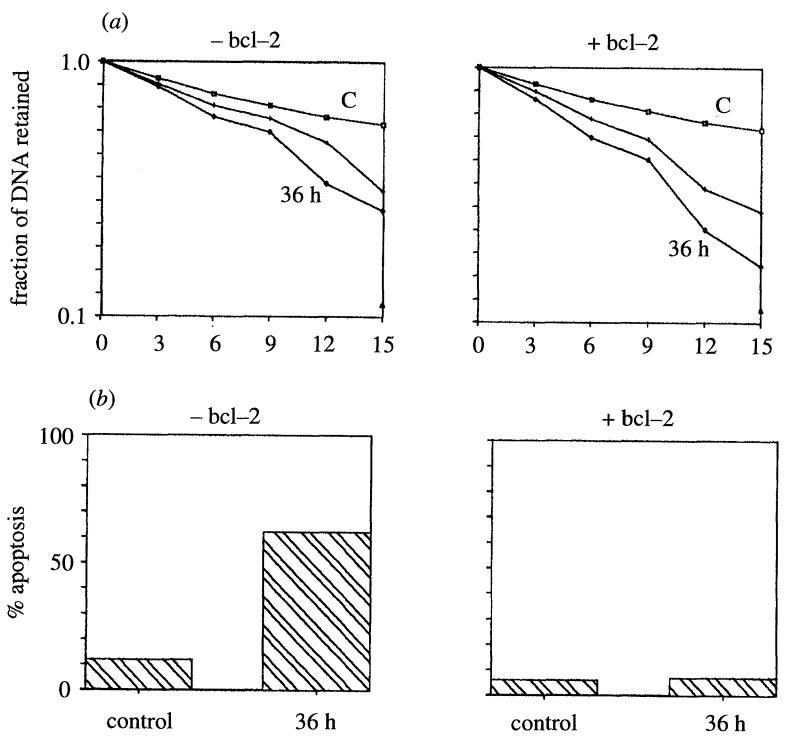

Figure 5. Composite showing (*a*) DNA strand breaks, measured by alkaline elution, in the nascent DNA of 5-fluorodeoxyuridine treated (1 μM for 24 h) human B lymphoma cells which had been transfected with either vector alone (left hand panels) or human bcl-2 (right hand panels) (C = control). (*b*) The percentage of apoptotic cells, measured by acridine orange fluorescence, 36 h after a 24 h treatment with 1 μM fluorodeoxyuridine. Modified from Fisher *et al.* (1993), used with permission.

(e) p53 is differentially expressed in the crypts of the small intestine and colon: further implications for chemotherapy of colonic tumours

In defining the accumulation of genetic changes that characterize the progression of colon cancer, the loss of function of p53, the so-called 'guardian of the genome' (Lane 1992), is observed to be a common, although possibly late event in tumour progression (Vogelstein *et al.* 1988). Amounts of cellular p53 have been observed to rise after DNA damage (reviewed by Lane 1993), and in mouse thymocytes from animals with a null p53 phenotype, created by homologous recombination, it was shown that DNA damage-induced apoptosis is a p53-dependent process, since the normal apoptotic response was lost in the absence of p53 (Lowe *et al.* 1993*b*; Clarke *et al.* 1993).

We have investigated the role of p53 in the apoptosis of stem cells in the crypts of the small intestine and colon (Merritt *et al.* 1994*a*). Immunohistochemical analysis of the amount and cellular distribution of p53 protein in the crypts before and after DNA damage caused by radiation is shown in figure 6. The result is congruent with previous observations about the positional distribution of apoptotic cells: p53 protein was elevated in the stem cell region of the crypts of the small intestine whereas in the colon, the response to damage was more muted and the appearance of p53 protein after irradiation of the animals was not coincident with the position of the colonic stem cells. Most importantly, as figure 6 shows, the expression of p53 was coincident with the position of apoptotic cells. To determine the role of p53 in apoptosis in the intestinal crypts we have also utilized the null p53 animals (Donehower *et al.* 1992). Deletion of p53 completely prevented DNA damage-induced cell death in intestinal crypts: in the small intestine of wild-type animals, the number of apoptotic events observed in 200 half crypts, 4.5 h after 8 Gy of irradiation, was 397. In the null p53 animals it was 10 (Merritt *et al.* 1994*a*). Essentially, the loss of p53 provided complete resistance to DNA damage-induced cell death and recent experiments have shown that the stem cell population of these irradiated p53 null animals is able to repopulate the crypt.

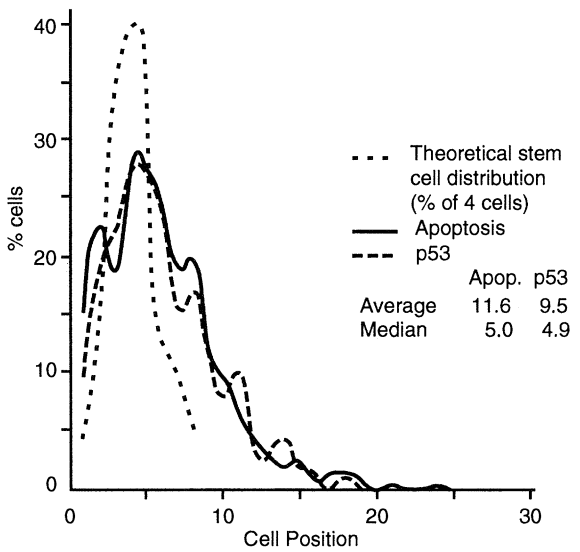

Figure 6. The relationship between the distribution of apoptotic fragments (solid line) and p53 positive nuclei (dashed line) along the length of the crypts of the murine small intestine 3 h after 8 Gy of irradiation. The theoretical positions of the stem cells, numbered from the base of the crypt, is also shown (dotted line). The average and median numbers of apoptotic cells was coincident with the expression of p53 protein.

Consequently, in terms of the genesis, progression and therapy of colonic tumours our data provide a bleak picture, reflecting the clinical reality of this disease. Stem cells of the colon, likely to be the fount of such tumours, are characterized by their low levels of spontaneous and induced apoptosis because, first, they selectively express bcl-2 and secondly they have a poor DNA damage response in terms of the expression of p53. The high survival potential provided by this phenotypic background may promote carcinogenesis and progression, because of the tolerance to further DNA damage. The muted p53 response in normal colonic stem cells is then lost in more than 50% of colonic tumours (Vogelstein *et al.* 1988). Moreover, some of these tumours maintain expression of bcl-2 (J. A. Hickman, C. S. Potten, A. J. Merritt & T. C. Fisher, unpublished data). Expression of bcl-2 provides resistance to drugs such as fluorodeoxyuridine, as described above, despite the imposition of considerable DNA damage. Cells with DNA damage of the type introduced by fluorodeoxyuridine, but which have lost p53, do not die (Lowe *et al.* 1993*a*).

3. FUTURE DIRECTIONS

The minimal impact of chemotherapy on disseminated colon cancer, and on other solid tumours, is frustrating to the pharmacologist and clinician. Its impact on the cancer sufferer is obvious. How might the impasse be broken? The ideas of stimulus–response coupling and of survival thresholds, described above, may offer avenues for progress. That said, some caveats are important: first, the central tenet of chemotherapy is that it should be selective. Secondly, due recognition must be given, as stated above, to the problem of directing a therapeutic agent to a single locus in a multistage disease. With these goals and cautions in mind, it seems appropriate to consider strategies which modulate the survival thresholds of tumour cells. Naively, one might suppose that reducing the effects of *bcl*-2 for a short time might allow DNA damage induced by standard drugs like 5-fluorouracil to initiate cell death in colonic tumours expressing bcl-2. But, toxicity to other normal tissues, normally protected by bcl-2 expression, would presumably be superimposed. What is required is a colon tumour cell-specific perturbation under conditions of a reduced threshold for cell survival. The nature of the target – the 'stimulus' – might not be critical. Most pertinently, the observation that non-dividing cells, such as post-mitotic neurons, may be prompted to engage apoptosis permits that proliferation biochemistry need not be

a target. The idea of an acceptable target redundancy would also overcome concerns regarding the question as to which of the multiple changes associated with the progression of malignancy should be targeted.

However, it is the nature of these changes themselves which might be the tumour cell-specific trigger for apoptosis. The astonishing feature of many tumours is their tolerance of genetic abberations, observed as changes in ploidy, chromosomal deletions and translocations. Attenuation of the capability to tolerate and survive genetic abberations may itself initiate apoptosis. The attractive idea of using the multiple lesions of carcinogenesis as the selective trigger for tumour self-destruction may not be too long in the testing.

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REFERENCES

Castle, V.P., Heidelberger, K.P., Bromberg, J., Ou, X., Dole, M. & Nunez, G. 1994 Expression of the apoptosis-supressing protein *bcl*-2, in neuroblastoma is associated with unfavourable histology and N-*myc* amplification. *Am. J. Path.* **143**, 1543–1550.

Clarke, A.R., Purdie, C.A., Harrison, D.J., Morris, R.G., Bird, C.C., Hooper, M.I. & Wyllie, A.H. 1993 Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature, Lond.* **362**, 849–852.

Collins, M.K., Marvel, J., Malde, P. & Lopez-Rivas, A. 1992 Interleukin 3 protects murine bone marrow cells from apoptosis induced by DNA damaging agents. *J. exp. Med.* **176**, 1043–1051.

Colombel, M., Symmans, F., Gil, S., O'Toole, K.M., Chopin, D., Benson, M., Olsson, C.A., Korsmeyer, S.J. & Buttyan, R. 1993 Detection of the apoptosis-suppressing oncoprotein *bcl-2* in hormone-refractory human prostate cancers. *Am. J. Pathol.* **143**, 390–400.

Dive, C. & Wyllie, A.H. 1993 Apoptosis and cancer chemotherapy. In *Frontiers in pharmacology and therapeutics: cancer chemotherapy* (ed. J. A. Hickman & T. R. Tritton), pp. 21–56. Oxford: Blackwell Scientific Publications.

Dive, C. & Hickman, J.A. 1991 Drug target interactions: only the first step in the commitment to a programmed cell death? *Br. J. Cancer* **64**, 192–196.

Donehower, L.A., Harvey, M., Slagle, B.L., McArthur, M.J., Montgomery, C.A. Jr, Butel, S. & Bradley, A. 1992 Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature, Lond.* **356**, 215–221.

Fisher, T.C., Milner, A.E., Gregory, C.D., Jackman, A.L., Aherne, G.W., Hartley, J.A., Dive, C. & Hickman, J.A. 1993 *bcl*-2 Modulation of apoptosis induced by anticancer drugs: resistance to thymidylate stress is independent of classical resistance pathways. *Cancer Res.* **53**, 3321–3326.

Foulds, L. 1958 The natural history of cancer. *J. Chronic Dis.* **8**, 2–37.

Goligher, J.C. 1980 *Surgery of the anus, rectum and colon.* London: Balliere Tindall.

Hickman, J.A. 1992 Apoptosis induced by anticancer drugs. *Cancer Metast. Rev.* **11**, 121–139.

Hockenbery, D.M., Zutter, M., Hickey, M., Nahm, M. & Korsmeyer, M.J. 1991 Bcl-2 protein is topographically restricted in tissues characterized by apoptotic cell death. *Proc. natn. Acad. Sci. U.S.A.* **88**, 6961–6965.

Ijiri, K. & Potten, C.S. 1987 Further studies on the response of intestinal crypt cells of different hierarchical status to eighteen different cytotoxic agents. *Br. J. Cancer* **55**, 113–123.

Ikegaki, N., Katsumata, M., Minna, J. & Tsujimoto, Y. 1994 Expression of bcl-2 in small cell lung carcinoma cells. *Cancer Res.* **54**, 6–8.

Kamesaki, S., Kamesaki, H., Jorgensen, T.J., Tanizawa, A., Pommier, Y. & Cossman, J. 1993 bcl-2 Protein inhibits etoposide-induced apoptosis through its effects on events subsequent to topoisomerase II-induced DNA strand breaks and their repair. *Cancer Res.* **53**, 4251–4256.

Lane, D.P. 1992 p53, guardian of the genome. *Nature, Lond.* **358**, 15–16.

Lane, D.P. 1993 A death in the life of p53. *Nature, Lond.* **362**, 786–787.

Leek, R.D., Kaklamanis, L., Pezella, F., Gatter, F. & Harris, A.H. 1994 Bcl-2 in normal human breast and carcinoma, association with ER positive EGFR negative tumours amnd in situ cancer. *Br. J. Cancer* **69**, 135–139.

Lowe, S.W., Ruley, H.E., Jacks, T. & Housman, D.E. 1993*a* p53-Dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* **74**, 957–967.

Lowe, S.W., Schmitt, E.M., Smith, S.W., Osborne, B.A. & Jacks, T. 1993*b* p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature, Lond.* **362**, 847–849.

McDonnell, T.J., Troncoso, P., Brisbay, S.M., Logothetis, C., Chung, L.W.K., Hsieh, J.-T., Tu, S.-M. & Campbell, M.L. 1992 Expression of the protooncogene *bcl*-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res.* **52**, 6940–6944.

Merritt, A.J., Potten, C.S., Kemp, C.J., Hickman, J.A., Balmain, A., Lane, D.P. & Hall, P.A. 1994*a* The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. *Cancer Res.* **54**, 614–617.

Miyashita, T. & Reed, J.C. 1993 Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* **81**, 151–157.

Pezzella, F., Turley, H., Kuzu, I., Tungekar, M.F., Dunnhill, M.S., Pierce, C.B., Harris, A.L., Gatter, K.C. & Mason, D.Y. 1993 *bcl*-2 Protein in non-small-cell lung carcinoma. *New Engl. J. Med.* **329**, 690–694.

Potten, C.S. 1992 The significance of spontaneous and induced apoptosis in the gastrointestinal tract of mice. *Cancer Metast. Rev.* **11**, 179–195.

Potten, C.S., Li, Q.Y., O'Connor, P.J. & Winton, D.J. 1992 A possible explanation for the differential cancer incidence in the intestine, based on distribution of the ctyotoxic effects of carcinogens in the murine large bowel. *Carcinogenesis, Lond.* **13**, 2305–2312.

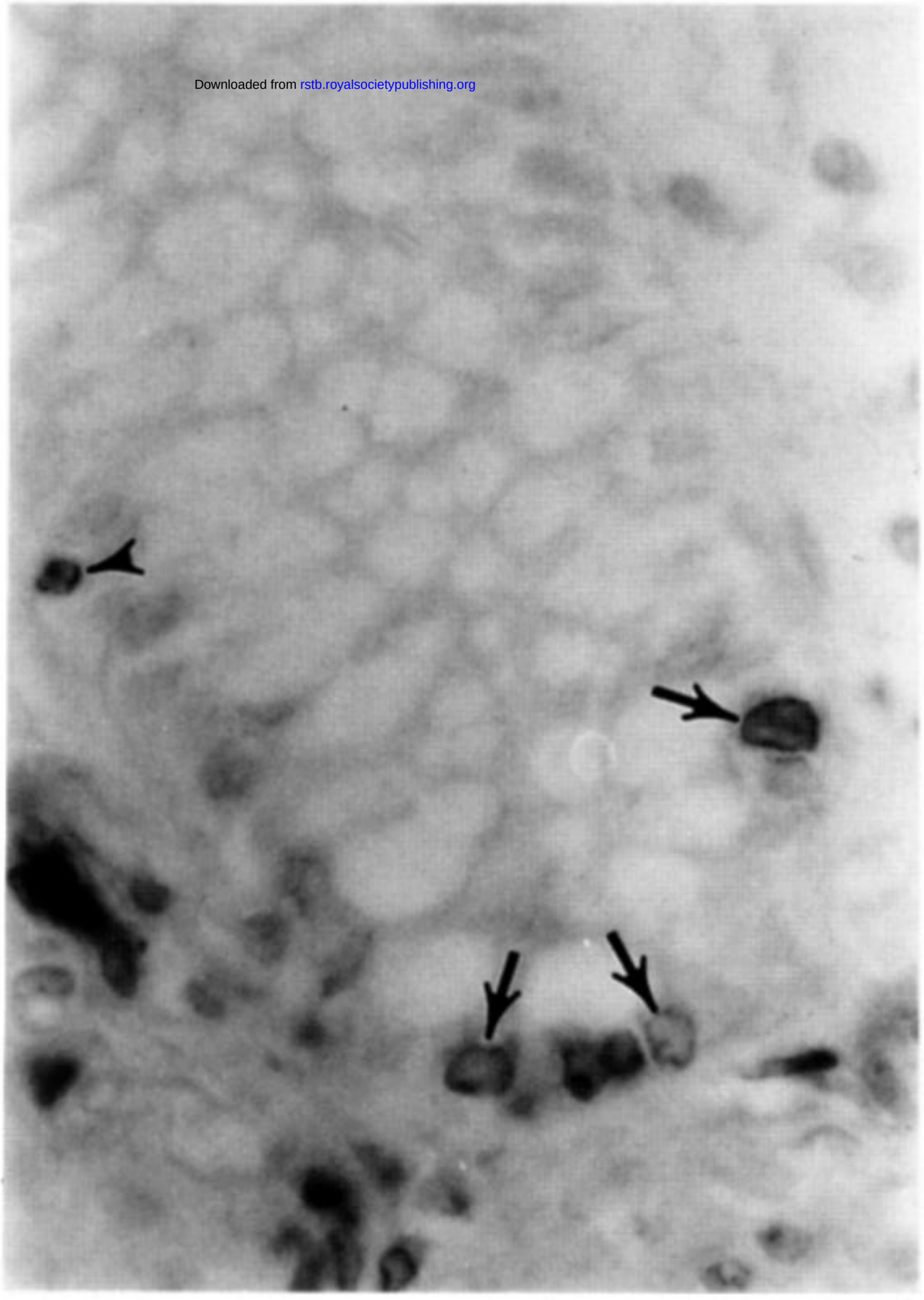
Reed, J.C. 1994 Bcl-2 and the regulation of programmed cell death. *J. Cell Biol.* **124**, 1–6.

Twentyman, P.R. 1992 mdr-1 (P-glycoprotein) gene expression: implications for resistance modifier trials. *J. natn. Cancer Inst.* **84**, 1458–1460.

Vogelstein, B., Fearon, E.R., Hamilton, S.R., Kern, S.E Preisinger, A.C., Leppert, M., Nakamura, Y., White, R., Smits, A.M.M. & Bos, L. 1988 Genetic alterations during colorectal tumour development. *N. Engl. J. Med.* **319**, 525–532.

Walton, M.I., Whysong, D., O'Connor, P.M., Hockenberry, D., Korsmeyer, S.J. & Kohn, K.W. 1993 Constitutive expression of human *Bcl*-2 modulates nitrogen mustard and camptothecin induced apoptosis. *Cancer Res.* **53**, 1853–1861.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



igure 3. Immunohistochemical analysis of the expression of
e human bcl-2 protein, by immunoperoxidase staining, in
normal colonic crypt.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

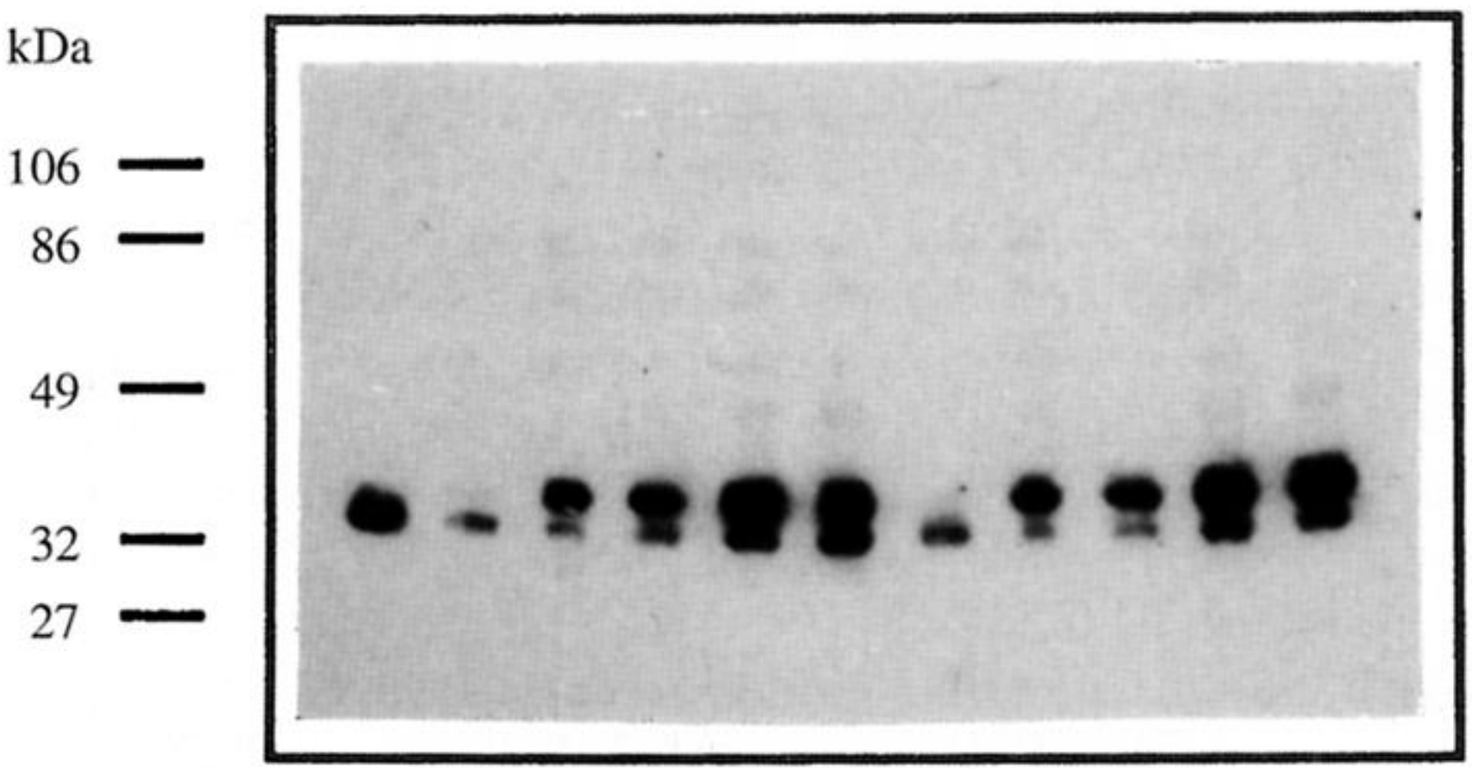


Figure 4. Western blot of the thmidylate synthase enzyme in human B-cell lymphoma cells transfected with ther vector (I Sv2) or human bcl-2 (I bcl-2). The blot shows changes in the amount of enzyme after various mes of incubation of the cells with 5-fluorodeoxyuridine, as the cells attempt to overcome the blockade of the nzyme. Also, with time, the blot shows the appearance of the higher molecular mass form of thymidylate nthase formed as the drug forms a ternary complex. (The lane WI-L2:c1 contains purified thymidylate nthase protein.) (From Fisher *et al.* (1993), used with permission.)