

## Correspondence

In the recent article by Krishan and Frei (9), the authors described striking morphological changes in cultured lymphoblasts undergoing cytolysis after exposure to *vinca* alkaloids, and it was suggested that these changes were characteristic of cell death induced by these particular drugs. It seems almost certain, however, that the phenomenon that they described was apoptosis (8), a recently defined mode of cell death that is morphologically quite distinct from the more familiar type of cell necrosis that occurs after disruption of normal plasma membrane function (13).

Typical apoptosis has been described during embryonic morphogenesis (10, 11), during anuran metamorphosis (4), in ischemic- (3) and endocrine-induced (7) atrophy of organs, and in untreated human carcinomas (5, 6). Characteristically, apoptosis affects scattered single cells that condense and bud off numerous membrane-bound fragments of cytoplasm that vary greatly in size. Some of these cells may contain nuclear remnants, but all contain crowded organelles that are initially well preserved. In solid tissues, apoptotic bodies are dispersed along intercellular planes before they are engulfed by neighboring cells, in which they are degraded in phagolysosomes (6).

During a recent *in vivo* study of the effects of single doses of actinomycin D (300 µg/kg), cytosine arabinoside (250 mg/kg), mitomycin C (3 mg/kg), and cycloheximide (300 mg/kg) on normal gut crypt epithelium and mouse Sarcoma 180 ascites tumor cells, it was found (by both light and electron microscopy) that the only form of cell death that occurred was apoptosis (12). The dispersal of the cells in the ascites tumor greatly facilitated the study of cellular fragmentation, the features of which were found to be the same as those illustrated by Krishan and Frei (9). The membrane-bound fragments that were formed were not phagocytosed, however, but remained floating freely, and although they appeared to be capable of some metabolic activity (1, 2), all ultimately underwent dissolution; at this stage they resembled apoptotic bodies that were seen in the lumens of prostatic glands after the onset of castration-induced atrophy (7).

Krishan and Frei (9) suggested that the morphological changes that they described were drug specific because a similar sequence of events was not observed when cells of the same line were exposed to bleomycin and adriamycin (the concentrations of which were not mentioned). Such comparisons may not be valid, for when different drugs are being used on the same cell system it is difficult to be sure that biologically equivalent doses have been administered. The results presented in their paper may indicate only that these latter drugs were less efficacious than were the *vinca* alkaloids and that the concentrations used were insufficient to cause any lymphoblast death within the allotted time.

Since several drugs (all with different modes of chemical action) have been shown to result in apoptosis in populations of proliferating normal and neoplastic cells (12), it would be unfortunate if such a characteristic and recogniza-

ble phenomenon were to be ascribed solely to the action of the *vinca* alkaloids. It is also important to realize that an ultrastructurally identical process occurs in many other situations in which enhanced cell deletion is occurring and that it can also be seen in normal tissues, where it appears to balance the increments in population caused by mitosis (8). The cause of apoptosis is not yet known, but it has been suggested that it is initiated by irreversible impairment of normal nuclear function (10, 12). By using cytotoxic drugs with known modes of action, more precise information may be gained about biochemical events involved in triggering this stereotyped sequence of cellular changes.

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Our paper (5) described, with the help of electron microscopy and time-lapse cinematography, the extreme sensitivity of CCRF-CEM human lymphoblasts (but not that of L-929 or HeLa cells) to the cytolytic effects of low concentrations of the *vinca* alkaloids, vinblastine and vincristine. We have also reported the formation and release of cytoplasmic vesicles from the peripheral cytoplasm of lymphoblasts exposed to vincristine or vinblastine. Similar morphological changes were not seen in either L-929 or HeLa cells exposed to these alkaloids or in lymphoblasts exposed to bleomycin (1 to 100  $\mu\text{g}/\text{ml}$ ) or adriamycin (1 to 10  $\mu\text{g}/\text{ml}$ ).

The phenomenon of "apoptosis" described by Kerr *et al* (1) and referred to by Dr. Searle is apparently similar to the phenomenon that we have described, and our *in vitro* time-lapse observations further show this to be an actual phenomenon rather than an artifact of fixation and tissue processing. As pointed out by Kerr *et al.* (1), apoptosis could be responsible for cell loss under various conditions of growth or in response to toxic agents.

In the last 2 paragraphs of his letter, Dr. Searle objects to our comparison of the effects of *vinca* alkaloids, adriamycin, and bleomycin without mention of their concentrations. However, in our paper (Ref 5; page 498, lines 8 and 9 from bottom) we did in fact indicate the concentrations of bleomycin (1 to 100  $\mu\text{g}/\text{ml}$ ) and adriamycin (1 to 10  $\mu\text{g}/\text{ml}$ ). These observations are further supported by our earlier published studies with time-lapse cinematography and electron microscopy or by the variety of morphological alterations seen in L-929 cells and CCRF-CEM lymphoblasts exposed to a wide concentration range of vinblastine,

vincristine (2, 3, 6, 7), bleomycin (4), or adriamycin (8).

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