The common occurrence of the diazo group in these two compounds suggests that this may be the active group causing this effect. We have shown,* that in other respects, this unsubstituted ω-diazoacetophenone, and several other meta and para substituted ω-diazoacetophenones do not have the actions of L-azaserine, in respect of its action in competing with L-glutamine in purine synthesis, or in having an antimitotic action on cell systems that are affected by L-azaserine. We therefore suggest that this common effect is caused by a mechanism distinctly different from that which operates when L-azaserine acts as an antagonist to L-glutamine in purine biosynthesis.

Although we are unable to suggest a site of action for this effect, we suggest that it is due to the possession of the diazo group on the molecule.

* Unpublished results.

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Stathmokinetic action of pentobarbital on cultured human kidney cells

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THE USE of synchronized cell cultures facilitates the investigation of the mode of action of pharmacological agents on cells by differentiating the susceptible phases of the cycle at which the compound is effective. Thus in the examination of mitotic poisons compounds which block division by interfering with DNA synthetic processes can be easily distinguished from compounds showing a true G_2 blocking action.

Furthermore a compound which blocks the onset of prophase can be distinguished from a stathmokinetic agent, such as colcemid, blocking in metaphase. By the use of cultures of human kidney cells, synchronized by the double thymidine blocking technique, it has been found that pentobarbital

(nembutal) at appropriate concentrations exerts a reversible stathmokinetic effect on these cells, eventually leading to nuclear pyknosis, which is not shown by several other barbiturates, and which appears not to have been previously reported.

Human kidney T-cells were cultured in LPC medium and synchronized by the double thymidine (TdR) blocking technique as previously described.[1] The inhibitors were dissolved in LPC medium immediately prior to use and the pH readjusted to 7·2 wherever necessary. Mitotic indices were counted from coverslip cultures. The biochemical effects of inhibitors on DNA, RNA and protein synthesis were determined for cells pulse labelled with thymidine-6-^{3}H, (1 μCi/ml), uridine-5-^{3}H, (0·5 μCi/ml), and L-histidine-2·5-^{3}H, (5 μCi/ml) for 30 min in the presence of the inhibitor.[2] Cells were grown as monolayers in T-flasks and were preincubated with inhibitor for 10 min prior to the addition of isotope. In the case of histidine pulsing, the medium contained only 1% of the normal lactalbumin content in order to avoid isotope dilution effects. The incorporation of precursor was estimated by liquid scintillation counting of an aliquot of the washed trypsinized cell suspension after dissolving in 0·3 N NaOH. After the removal of the 2nd TdR block from monolayer cultures

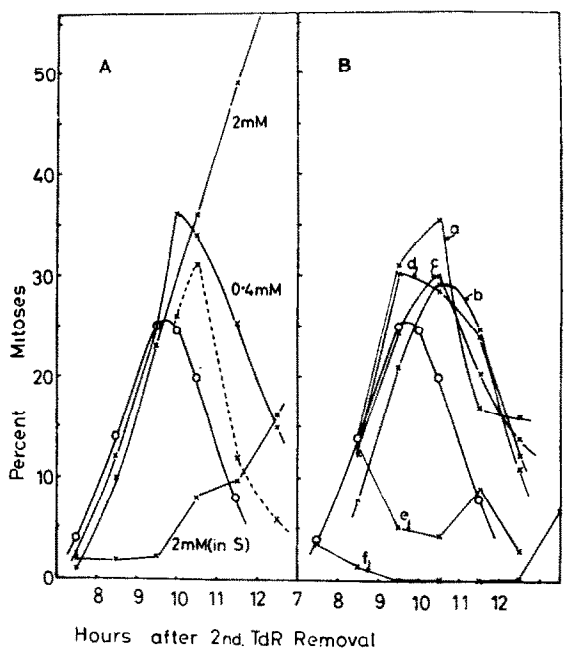

FIG. 1. Effect of barbiturates, cycloheximide and pederin on mitosis in synchronized human kidney T-cells. Second thymidine block was removed at 0 hr. Other times are hours post-TdR block.
A. Pentobarbital added in S (2·5 hr) or in G_2 (7 hr). ○—○ control. × · · · · × pentobarbital (2 mM) added at 7·5 hr and washed off cells at 9·5 hr.
B. Barbiturates added at 7 hr.
a, phenylbarbital 5 mM; b, amylobarbital 2 mM; c, cyclobarbital 2 mM; d, diethylbarbital 10 mM; e, cycloheximide 10μg/ml added at 8 hr; f, pederin 0·002 μg/ml added at 7 hr.

a synchronous wave of division occurs in normal cells with a peak value of 22–25% mitotic figures at 9·5 hr later. The effect of pentobarbital on this synchronous wave of division is seen in Fig. 1A. When added to cells in G_2 (7 hr) at a concentration of 2 mM, the mitoses continue to accumulate at an approximately linear rate until well over 50% of the cells are blocked in metaphase. With prolonged incubation the chromosomes show progressive ball-clumping and eventually become pyknotic. This pyknosis in the case of colchicine has been attributed to the depletion of essential *m*-RNA in

animal cells.[3] The appearance of the cells (lack of spindle structure) and their subsequent behaviour closely resembles that of these cells when blocked by colchicine or colcemid in the range 0·02–0·5 μg/ml. T-cells are particularly sensitive to these agents. The block appears to be completely reversible however (like that of colcemid) for treatment periods of up to 2 hr, at this concentration of 2 mM. At lower concentrations, 0·4 mM or less, the block is not complete and the cells eventually progress through division. Higher concentrations, 4 mM or greater, are toxic to the cells and block the initiation of prophase and subsequently lead to degeneration of the cells. When added to cultures in the DNA-synthetic S-phase pentobarbital delays the initiation of mitosis presumably by reason of its action on DNA synthesis, as is shown in Table 1 and has been reported for other cells.[4]

TABLE 1. EFFECT OF BARBITURATES, CYCLOHEXIMIDE AND PEDERIN ON RNA, PROTEIN AND DNA SYNTHESIS IN HUMAN KIDNEY T-CELLS

Compound	Percentage of control		
	RNA	protein	DNA
Control (c/m/10^6 cells)	58,700	42,500	7,200
Pentobarbital (2 mM)	8·2	29	33
Amylobarbital (2 mM)	9·0	88	33
Phenylbarbital (2 mM)	60	93	69
Cyclobarbital (2 mM)	19	94	53
Diethylbarbital (2 mM)	72	92	76
Pederin 0·002 (μg/ml)	105	61	—
Pederin 0·01 (μg/ml)	108	6	—
Cycloheximide (10 μg/ml)	99	12	—
Puromycin (2 μg/ml)	102	65	—

Inhibitors were added to monolayer cultures of T-cells for 10 min prior to the addition of labelled precursor. DNA synthesis was measured with ^{3}H-TdR (1 μc/ml), RNA synthesis with uridine-5-^{3}H (1 μc/ml), and protein synthesis with L-histidine, 2-5-^{3}H (5 μc/ml), for 30 min followed by washing and trypsinization of the monolayers. Results are the mean of two separate experiments for each determination.

The action of several other barbiturates under the same conditions is shown in Fig. 1B. Even at higher concentrations (up to 10 mM in one case) no evidence of any significant stathmokinetic effect was obtained. A transient effect is, however, shown by 2 mM amylobarbital. Higher concentrations of amylobarbital and cyclobarbital are precluded by solubility limitations. A similar lack of effect of amylobarbital on mitosis has been reported for chinese hamster cells.[5] By contrast with these results, the rapid block of the initiation of prophase in these cells, shown by the specific protein blocking agents cycloheximide and pederin[6] is evident (Fig. 1B).

Previous studies with these cells[1, 7] and others[8–10] have indicated that protein and RNA synthesis are required until almost the initiation of prophase, although in HeLa cells this requirement is apparently less rigid.[11] Since in these cells cycloheximide and pederin do not affect RNA synthesis at concentrations at which they strongly inhibit amino acid incorporation (Table 1), the initiation of cell division is dependent upon the synthesis of specific division proteins. These proteins can be made non-functional by the incorporation of unnatural amino acid analogues.[7] Puromycin (2 μg/ml) was found to have only a weak (35 per cent) inhibitory action on amino acid precursor incorporation into T-cells but in view of its mode of action, this incorporation is possibly not into fully formed protein chains but into non-functional polypeptides since like the above two inhibitors, it also is a powerfu G_2 blocking agent.[1]

In this respect it is significant that pentobarbital exerts the most powerful inhibitory action on both RNA and protein synthesis of all the compounds tested. Amylobarbital, the only other compound showing a tendency to a stathmokinetic effect also affects RNA synthesis strongly (Table 1). The inhibitory effect on DNA synthesis, although marked, can be disregarded as a mode of action

since the effects are clearly manifest in the G_2 phase of the cycle, after all DNA synthesis is finished. It is doubtful, however, whether the inhibitory action of pentobarbital on protein synthesis can explain its stathmokinetic blocking action, in view of the different behaviour of cells blocked from entering prophase by cycloheximide and pederin. The G_2 blocking action of higher concentrations of pentobarbital may, however, be due to this inhibitory effect. The morphological similarity to cells treated with colchicine and colcemid suggest that a similar mode of action may be involved[12] or else some other interference with the mitotic spindle as in the case of rotenone.[5]

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Uncoupling of oxidative phosphorylation by arylhydrazono-isoxazolone fungicides

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4-ARYLHYDRAZONO-3-METHYL-5-ISOXAZOLONES are effective fungicides against a number of plant pathogens[1, 2] but little is known of their mode of action. In view of their structural similarity to arylhydrazonomalononitriles, which are potent uncouplers of oxidative phosphorylation,[3, 5] it has been suggested[2] that the isoxazolone fungicides may also exert a similar uncoupling effect. To test this theory we have examined 3-methyl-4-phenylhydrazono-5-isoxazolone (I;X = 0, R = H), the *o*-chlorophenyl analogue known as drazoxolon (I;X = 0, R = 2 − Cl) and the *m*-chlorophenyl derivative (I;X = 0, R = 3 − Cl) as uncouplers of oxidative phosphorylation of rat liver mitochondria in comparison with 2,4-dinitrophenol. We included in the tests three structurally related compounds which are generally much less fungitoxic,[2] namely, 3-methyl-4-phenylhydrazono-5-pyrazolone (I; X = NH, R = H), the 1-phenyl analogue (I; X = N.Ph, R = H) and 4-benzylidene-3-