Inhibitory action of aspirin on DNA synthesis in phytohaemagglutinin-stimulated human lymphocytes

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The effects of drugs on blastogenesis in lymphocytes cultured with the mitogen phytohaemagglutinin (PHA) have received much attention because this phenomenon is accepted as an *in vitro* reflection of *in vivo* response, and has been used as an indicator of some diseases (Rubin, 1970; Elves, 1972). The transformed lymphocytes have morphological characteristics of primitive cells, can enter the proliferative cell cycle and within 24–48 h begin to synthesise DNA. Incorporation of tritiated thymidine (^{3}H-TdR) is often used to estimate the rate of transformation since peripheral lymphocytes must undergo blastogenesis before DNA synthesis can occur and such cells must pass through the S-period *in vitro* before mitosis. However, the presence of a drug which inhibits DNA synthesis could clearly affect results or cause misinterpretation of data in experiments carried out to estimate lymphocyte transformation solely by ^{3}H-TdR uptake. Similarly, the influence of such a drug on mitosis is not easy to assess directly.

Drug-mediated inhibition of DNA synthesis in cultured lymphocytes has been demonstrated, e.g. by diphenylhydantoin (Mackinney & Vyas, 1973), rifampicin (Nilsson, 1971), and phenylbutazone and oxyphenbutazone (Dewse, 1976). These observations suggest that some agents previously reported as depressing lymphocyte transformation, e.g. aspirin (Opelz, Terasaki & Hirata, 1973; Dam, Malkinson & Gewurz, 1975), or mitosis, e.g. phenylbutazone (Breull & Karzel, 1970; Wismüller, 1971) and chloramphenicol (Pisciotta & DePrey, 1967; Nasjletti & Spencer, 1968) may rather cause inhibition of DNA synthesis. Uptake of ^{3}H-TdR and the incidence of mitoses would then necessarily be reduced. Results obtained from experiments to investigate the effect of one such drug, aspirin, on thymidine incorporation in proliferating PHA-stimulated human lymphocytes illustrate the possibility of misinterpretation of results from experiments designed to show the immunosuppressive action of pharmaceutical agents by measurement of depression of ^{3}H-TdR uptake only.

Replicate suspensions of PHA-stimulated lymphocytes after 3 days in culture were incubated with ^{3}H-TdR and different concentrations of aspirin (30, 150, 300, 500 μg ml^{-1}) for 1 h at 37°, after which thymidine uptake was estimated. Techniques described previously were followed in lymphocyte culture (Dewse, 1976) and in estimating ^{3}H-TdR incorporation by TCA precipitation of DNA on fibreglass discs and liquid scintillation counting (Dewse & Potter, 1975). Assays were done in duplicate on 11 blood samples from 9 healthy, unmedicated subjects, 6 males and 3 females. Means of the duplicate values were expressed as percentage uptake in parallel cultures incubated with no drug (controls), and are given in Table 1. The overall variance (s^2) of these duplicates and controls was $\pm$ 12·16, and the similarity of the results ($s^2 = \pm$ 15·46) in the repeat samples from 2 subjects, I and II, shows that reproducibility is good.

In each instance some reduction in ^{3}H-TdR uptake was caused by aspirin at the standard therapeutic plasma concentration of 300 μg ml^{-1} ($\equiv$ 30 mg %; range: 85·94–19·46; mean $\pm$ s.e.m. = 53·68 $\pm$ 6·09). More severe inhibition was caused by aspirin at 500 μg ml^{-1} (range: 75·28–3·89; mean $\pm$ s.e.m. = 31·54 $\pm$ 7·13), where thymidine uptake was reduced to less than 20% of control uptake in cells from 5 of the 9 subjects. With aspirin at the lower concentrations of 30 and 150 μg ml^{-1} the lymphocytes of 5 of the subjects showed inhibition of ^{3}H-TdR, with reductions approaching 50%, but the cells from the other 4 subjects showed thymidine uptake elevated to some 20% above controls. It should be noted that this elevation, which compares with that found with phenylbutazone at low concentrations (Dewse, 1976), occurred in samples from males, but the significance of this observation cannot be assessed from these data.

Morphological examination of cytocentrifuge preparations made from the cultured cells revealed blastoid cells and mitotic figures in each case, and this evidence in conjunction with the incorporation of thymidine in DNA synthesis shows active proliferation in the cells upon which the assays were performed. These results

Table 1. *Influence of short-term exposure to different concentrations of aspirin on uptake of tritiated thymidine by PHA-stimulated human lymphocytes* in vitro. Mean values of duplicate assays expressed as percentage uptake in controls.

Subject	Sex	Aspirin concn (μg ml^{-1}) 30	150	300	500
I_1	F	83·80	56·70	45·60	26·30
I_2	F	72·40	51·70	50·90	29·60
II_1	M	114·56	117·56	78·20	54·37
II_2	M	not done	113·61	75·71	55·24
III	M	100·45	100·00	85·94	75·28
IV	M	93·20	60·90	19·46	10·80
V	M	109·80	102·80	62·30	8·02
VI	F	79·27	76·71	40·03	17·07
VII	F	90·22	65·49	41·21	3·89
VIII	M	83·76	61·60	36·50	15·80
IX	M	120·10	88·10	54·64	50·59
Mean		94·75	81·38	53·68	31·54
$\pm$s.e.m.		$\pm$5·05	$\pm$7·24	$\pm$6·09	$\pm$7·13

therefore show that DNA synthesis can be inhibited in proliferating PHA-stimulated lymphocytes by short-term exposure *in vitro* to therapeutic concentrations of aspirin. Such inhibition would perforce be immunosuppressive in that depression of DNA synthesis would lead to reduction in proliferative response, and thus both ^{3}H-TdR incorporation and also mitosis would fall. This mechanism may explain the lowered growth rate *in vitro* of human embryonic cells caused by the aspirin metabolite, sodium salicylate (Paine & Nagington, 1971).

When DNA synthesis was inhibited by 5-fluorodeoxyuridine (Salzman, Pelegrino & Franceschini, 1966) and also when mitosis was reported to be affected directly by chloramphenicol (Pisciotta & DePrey, 1967; Nasjletti & Spencer, 1968), the degree and quality of morphological transformation of PHA-stimulated lymphocytes has been described as unchanged. Conversely, Caron (1967) expressed doubts about these results and found no blastoid cells when ^{3}H-TdR uptake was completely stopped *in vitro* by methotrexate. However, the degree of blastogenesis represents potential proliferation whereas incorporation of thymidine is a measure of active DNA synthesis and therefore of actual proliferation. These are two different phases of immunological response and may be affected independently by immuno-suppressive agents (Bradley & Elson, 1971). Failure to distinguish between the different phases of the proliferative response may lead to spurious results and may obscure the mode of action of some immunosuppressive and pharmacological agents.

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Enterohepatic circulation of sodium sulisatin* and its effects on glucose absorption in the rat

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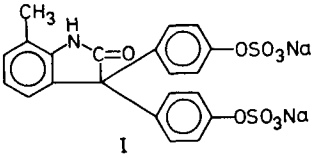
Synthetic laxatives with two phenolic groups either free or esterified with acetate or sulphate may undergo biliary elimination (Ferlemann & Vogt, 1965; Vogt, Schmidt & Dakhill, 1965; Fingl, 1975; Smith, 1966; Jauch, Hankwitz & others, 1975). We have studied the enterohepatic circulation of sodium sulisatin [disodium salt of sulphuric diester of 3,3-bis-(4-hydroxyphenyl)-7-methyl-2-indolinone, DAN-603, I], a new laxative whose activity and pharmacological properties have been reported recently (Garrido, Ibáñez & others, 1975; Moretó, Goñalons & others, 1976).



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