time output function results from the 2 input functions. The force increases curvilinear with an overproportional heightening of the force amplitudes. In the dynamical relaxation phase, we observe in figure 4 a pronounced upper and a slight lower summit decrease. The force difference of the upper and lower summit decreases initially. This appearance is called amplitude (or heave) diminution phenomenon of the dynamic relaxation in the lower nonlinear part of the extension-time curve. Figure 5 shows after a fast stretch under constant length and superponed sinus wave extensions a short dynamic relaxation phase. After a repeated partial relieve of the tension on account of an induced decrease of the length and repeated interposition of isometric conditions, an initial increase of the force amplitudes can be observed (figure 5). This appearance is called inverse dynamic (cyclic) relaxation or dynamic (cyclic) force recovery. The amplitudes of the sinus wave strain input function can be seen in the upper part of figure 5. The amplitudes of the output function recovery curves are small in the lower force regions.

*Discussion.* The main component of the liver capsule are collagen fibres. They have biomechanical functions[14]. The considerable initial compliance with a high resistance force under further elongation is an important protective mechanism with blunt abdominal injuries. The substantial elongation properties of the liver capsule at the beginning of the force-extension or force-time diagram are essential in acute volume expansions of the liver in as much as the tissue pressure of the parenchyma increases protacted initially.

The histomechanical diagrams are reproducible provided that a series of identical curves in considerations of equal time distributions are generated, and on the condition that we take into consideration the curves of the steady state phase after the transient phase, for example the last cycles of figure 3. Figure 2 represents a curve out of a series in the steady state phase. After a partial force release, the liver capsule does not contract instantaneously like an ideal elastic body because the elastic reset forces are damped by viscous elements of the tissue[15]. Under isometric conditions (constant length) after a partial force or length release, a time dependent static or dynamic (cyclic) force recovery arises.

Our experiments have shown a complex mechanical behaviour of the liver capsule. The biomechanical properties of the liver are evidently versatile and adapted to physiological and pathological stress-strain conditions and within certain limits to traumatic impacts[16].

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## Sister chromatid exchanges in human lymphocytes exposed to 8-methoxypsoralen and long wave UV radiation prior to incorporation of bromodeoxyuridine

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*Summary.* Human lymphocytes exposed to the effects of long wave UV radiation in the presence of 8-methoxypsoralen prior to stimulation by PHA show dose related sister chromatid exchanges after 2 replication cycles in vitro. This has implications for interpreting the repair processes involved and for monitoring DNA damaging agents in vivo.

8-Methoxypsoralen (8-MOP) is a furocoumarin compound which, on being excited by 360 nm radiation, forms covalently bound adducts with pyrimidines[1]. The molecule may form adducts at either or both ends so that monoadducts and cross links affecting both strands of the DNA molecule are formed[2]. Clinically it has been used in the treatment of psoriasis[3] and suggestions that the treatment may cause chromosome damage in man[4] and be potentially mutagenic or carcinogenic have given rise to concern.

The recently developed techniques[5,6] for staining chromosomes after cells have been grown for 2 rounds of replication in the presence of bromodeoxyuridine (BrdU), which substitutes for thymidine, make it possible to distinguish between chromatids in which both DNA strands have been substituted and those retaining the original 'old' DNA strand. This has permitted the clear demonstration of sister chromatid exchanges (SCEs) when these occur[7]. A number of known carcinogens and mutagens when present in the culture medium have been shown to have marked effects on the rate of SCE in cultured chinese hamster cells[8], or human leukocytes[9], presumably because these substances cause damage to the DNA.

We have used the BrdU technique, essentially as described by Perry and Wolff[6] with minor modifications, to examine the effect of 8-MOP and long wave UV radiation (UV-A 315–390 nm) on the production of SCEs in human lymphocytes in vitro when the treatment is administered prior to the addition of BrdU to the cultures. This has shown that the effect of certain DNA damaging agents in producing SCEs can be demonstrated without the necessity of labelling the chromosomes before the administration of the agent.

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*Material and methods.* Heparinized blood samples were obtained by venepuncture from 13 healthy adults. 0.4 ml of whole blood was added to 4 ml of culture medium (85% Eagles medium and 15% calf serum with 100 units $ml^{-1}$ penicillin and $1 \times 10^{-4}$ mg $ml^{-1}$ streptomycin) and subjected to the appropriate experimental procedure, after which the cells were transferred to universal containers which were placed in a light-tight box. Each culture was then injected, through the lid of the box, with 0.1 ml phytohaemagglutinin and BrdU to give a final concentration of 12 μM and then incubated for 72 h. Colchicine (0.002% w/v) was added for the last 3 h of culture life to accumulate mitoses. Chromosome preparations were made and stained with 0.7 μg/ml Hoechst 33258 and Giemsa[6].

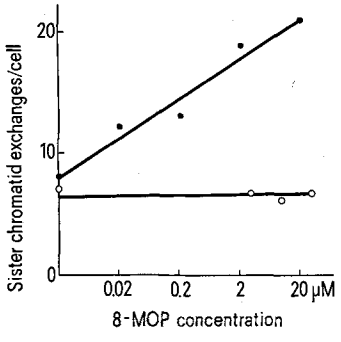


Fig. 1. The effect of 8-MOP concentration in inducing sister chromatid exchanges after 72 h culture without UVA treatment (○) and with 30 min irradiation of the 8-MOP treated blood by 2 parallel Atlas 20 W fluorescent UV (non-filter) tubes which have an emission peak at about 350 nm and negligible irradiation below 310 nm (●). The 2 tubes were fixed 14 cm apart and the flasks (80 $cm^2$ NUNC tissue culture flasks) containing the cells were placed beneath the tubes at a distance of 14 cm. The intensity of irradiation received by the cells measured by thermopile was 0.41 $mW/cm^2$. Each point on this figure and in figure 3 represents the mean of between 30 and 150 cells.

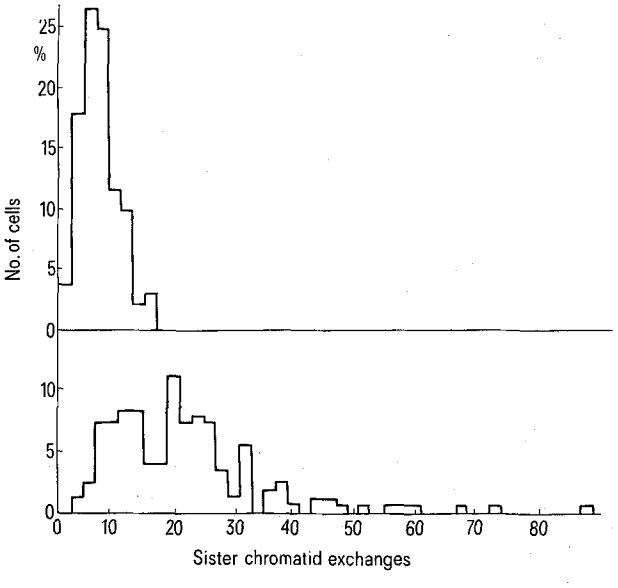


Fig. 2. The distribution of SCEs in cells (n = 155) from the control cultures (above) and in cells (n = 149) from cultures treated with 20 μM MOP and subsequently to 30 min exposure to UVA (below).

All slides were coded so that they could be scored 'blind'. Satisfactory mitoses for scoring were selected. Criteria used for distinguishing satisfactory mitoses were a) good differential staining of chromatids; b) a count of 46 chromosomes; c) absence of overlaps between chromosomes which could not be readily interpreted. The number of sister chromatid exchange points (SCEs) in each cell was recorded and a minimum of 30 cells were scored from each culture.

*Results and discussion.* Initial investigations showed that 8-MOP added to the cultures in concentrations up to 40 μM had no appreciable effect on the rate of SCE but when the cells, 15–30 min after the addition of 8-MOP, were irradiated with UV-A increase in 8-MOP concentration was reflected in an increase in the mean SCE rate (figure 1). At concentrations above 20 μM of 8-MOP, with irradiation for 30 min, difficulty in finding mitoses in the second division was encountered due to toxic effects or DNA replication delay caused by the treatment. No attempt has been made in these experiments to alter the culture time to take account of the effects of such delay although it is probable that delayed cells have a higher SCE frequency. The relationship found between SCE and log 8-MOP concentration shown in figure 1 is based on the number of SCEs found after 72 h of culture and may represent an underestimate, at higher concentrations, of the number of SCEs induced by the treatment. That this is so is further indicated by the findings of Perry and Evans[8] who adjusted their sampling time to give high yields of cells in the second division and were able to obtain considerably higher SCE frequencies at high concentrations of the active drugs which they tested. A comparison between the distributions of SCEs per cell in the control cultures and cultures which have been treated with 8-MOP and UV shows that the increase in SCE rate with increase in 8-MOP concentration is due not to an increased rate for all cells in the population but only in a part of it. This may be due to genuine differences in cellular sensitivity or to limitations imposed by the experimental system used as overlaying of one cell by another shields some cells from the light, a problem not encountered with monolayers of surface anchored cells. The effect is shown in figure 2 for an 8-MOP concentration of 20 μM. The proportion of cells with SCEs in excess of 18 was found to be 10% at 0.02 μM, 21% at 0.2 μM, 36% at 2 μM and 57% at 20 μM.

The effect of variation in UV-A exposure on the SCE rate is shown in figure 3. Increasing exposure to UV-A alone, at an irradiance of 0.41 $mW/cm^2$ does raise the SCE rate by a small amount. In the presence of 20 μM 8-MOP, in-

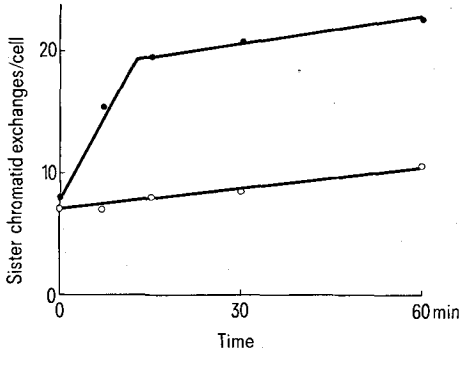


Fig. 3. The effect of varying the length of time of irradiation on SCE. The conditions are as described for figure 1. Irradiation alone was examined (○) as well as irradiation at an 8-MOP concentration of 20 μM (●).

creased SCE rates are obtained with increasing exposure times up to about 15 min exposure after which there is no further significant increase other than that due to UV-A alone. With the other concentrations of 8-MOP used in these experiments this plateau in SCE rate, the level of which is dependent on the 8-MOP concentration, was also reached within 15 min.

An interesting feature of these investigations, unlike those studies in which an active agent is present throughout[7] or during the latter half of culture life[8], is that the damage must occur on exposure to the light in $G_0$ when the chromosomes have not incorporated BrdU label into either strand. Nevertheless, there is a dose related response in terms of visible SCEs after 2 replication cycles. Preliminary experiments (not shown) in which BrdU was added later (24 h after the initiation of culture) indicate that incorporation of BrdU during repair immediately after 8-MOP/UV-A treatment does not account for the induction of the SCEs. Mitomycin C which, like 8-MOP with UV-A, produces DNA strand cross-links[10] is also a potent inducer of SCEs[7,8] suggesting this form of damage leads to SCEs. However, these agents produce monoadducts as well as cross-links[11] and it may be that it is single strand damage which results in SCEs while much of the damage due to cross-links is unrepairable and lethal to these cells. The lethality of these drugs would support this interpretation. In any case it would seem that some of the damage remains unrepaired until after replication although we have no information on what proportion this may represent. Post-replication repair is believed to be error prone[12] and if SCEs reflect this form of repair their appearance should be regarded as an indication of an increased risk of mutation.

A recent report[13] described a study of SCE rates after 8-MOP and UV-A in cells from a single healthy adult. A precise comparison is difficult because of differences in irradiation technique used but an 8-MOP concentration dependent increase in SCEs was also shown.

It is of practical importance that damage caused to lymphocytes in $G_0$ by UV activated 8-MOP results in a dose dependent increase in SCEs because it follows, as we have been able to demonstrate[14], that blood taken from patients treated with 8-MOP and irradiated in vitro should show SCE rates which would indicate the level of 8-MOP in the circulation. Further it should be possible to assess damage which occurs in vivo provided the cells have not replicated since the damage occurred. We are currently investigating this possibility.

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## Cytochemical localization of the $K^+$ regulation interface between blood and brain

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*Summary.* Phosphatase activity identified with $Na^+$-$K^+$-ATPase was localized at the basal surface of cerebral cortical capillary endothelium by perfusion with a p-nitrophenyl phosphate-strontium medium. The relationship of this to the blood-brain barrier to $K^+$ is discussed.

The concentration of $K^+$ in cerebrospinal fluid and brain intercellular fluid is maintained at a level below that of normal blood plasma, and is largely independent of fluctuations in plasma $K^+$ concentration[1]. The maintenance of this concentration difference implies the presence of an active transport system capable of expelling $K^+$ from brain intercellular fluid into blood. 2 groups of structures seem to be possible sites of active regulation: the endothelium of brain capillaries, and the astrocytes whose foot processes terminate on the basal lamellae of these capillaries[2]. The close proximity of these sites means that an ultrastructural approach is required for discrimination between them. We have examined this problem using a cytochemical technique, based on the capture by $Sr^{2+}$ of phosphate liberated from p-nitrophenyl phosphate, for the localization of the phosphatase step of $Na^+$-$K^+$-ATPase[3].

*Method.* The brains of adult male rats were fixed by perfusion for 15 min at 15 °C with a fixative at pH 7.4 containing glutaraldehyde, 0.25%; formaldehyde, 2.0%; dimethyl sulphoxide, 5.0%; sodium cacodylate, 0.1 M. The brain was then perfused in situ with the following solutions; the time intervals indicate equilibration periods between perfusions: 0.1M Tris-HCl pH 9.0, $2 \times 2$ min; incubation medium, $4 \times 20$ min; 0.1M Tris-HCl pH 9.0, $2 \times 10$ min; 0.25M sucrose, $1 \times 1$ min; 1.0% $Pb(NO_3)_2$, $2 \times 5$ min; 0.25M sucrose, $1 \times 2$ min; 0.1 M Tris-HCl pH 9.0, $2 \times 5$ min (composition of incubation medium: Tris-HCl pH 9.0, 100 mM; $MgCl_2$, 10 mM; $SrCl_2$, 20 mM; KCl, 10 mM; disodium p-nitrophenyl phosphate, 5 mM; L-tetramisole, 1 mM)[4]. Pieces of temporal cortex were post-fixed in 1% osmium tetroxide and prepared for electron microscopy.

*Results and discussion.* Electron-dense reaction product appeared as small, granular clumps on plasma membranes. The bulk of the product was found on the surfaces of the capillary endothelial cells remote from the blood (figure 1). Reactions elsewhere were weaker or absent. Control brains, perfused with either $K^+$-free or substrate-free media, showed little or no reaction product (figure 2). These experiments show the presence of a $K^+$-dependent p-nitrophenyl phosphatase activity of the type identified with the phosphatase step of $Na^+$-$K^+$-ATPase in the kidney and other organs[4-7]. We have previously shown that

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