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, 2×2 min; incubation medium, 4×20 min; 0.1M Tris-HCl pH 9.0, 2×10 min; 0.25M sucrose, 1×1 min; 1.0% $Pb(NO_3)_2$, 2×5 min; 0.25M sucrose, 1×2 min; 0.1 M Tris-HCl pH 9.0, 2×5 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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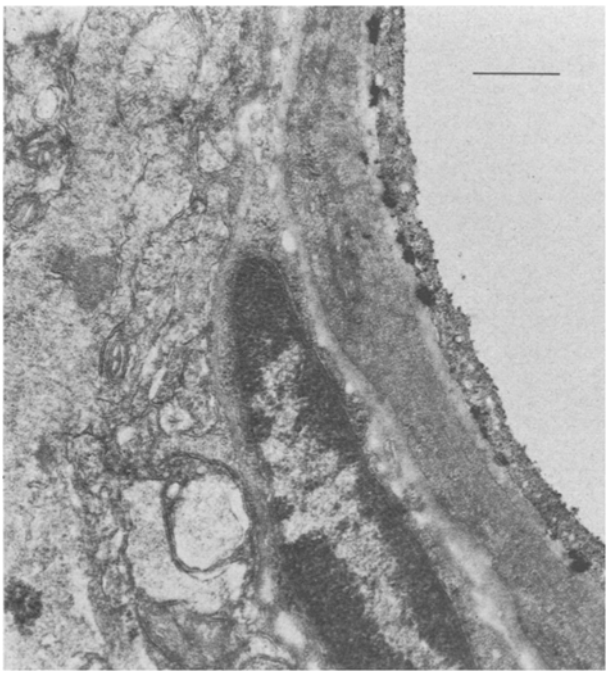

Fig. 1. Incubation in presence of 10 mM K^+. Electron-dense reaction product present on basal surface of endothelial cell. Pericytes and glial processes free of reaction. Bar represents 0.5 μm.

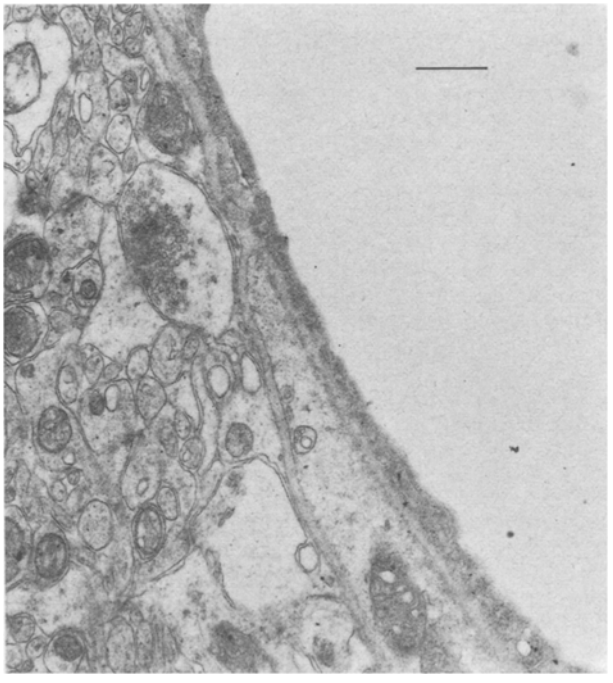

Fig. 2. Incubation in K^+-free medium. Reaction weak or absent in all layers. Bar represents 0.5 μm.

such activity is well preserved in rat brain under the fixation conditions employed here, and is not seriously inhibited by the components of the incubation medium, either singly or together[8]. However, the possibility exists that the endothelial localization is determined by the vascular route of presentation of media rather than by the true anatomical distribution of the enzyme. Presentation of incubation media by perfusion may bias the intensity of reaction so that sites near the vessel lumen appear most active. Unfortunately this is difficult to test as incubation of perfusion-fixed 50 μm slices of brain in vitro gives a widely distributed reaction independent of K^+.

The evidence presented supports the proposal that K^+ concentration in brain intercellular fluid may in part be regulated by active transport through endothelial cells, mediated by Na^+-K^+-ATPase at endothelial plasma membranes. This does not exclude the possibility of glial participation, as the large surface area of the glial processes could compensate for their apparently low Na^+-K^+-ATPase activity per unit area. However, it is probable that K^+ transport at the blood-brain interface depends significantly on the activities of the endothelium, in contrast with the epithelium-dominated transport processes of the choroid plexus[9, 10].

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14-3-2 Protein in rat brain synapses[1]

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Summary. The distribution of the 14-3-2 protein in rat brain synapses was studied by immuno electron microscopy. The protein was localized to the postsynaptic web and to the postsynaptic membrane, but was also prominent both in the presynaptic membrane and in the presynaptic densities. No significant activity was observed in the synaptic vesicles.

The distribution of the 14-3-2 protein, isolated and characterized by Grasso et al.[2], was studied by immunoelectron microscopy. 14-3-2 is a neuronal protein with a mol.wt of about 48,000–50,000[2]. The accumulation of the protein during postnatal development of the mouse and the avian optic tectum has been studied[4, 5], and it was found to accumulate during the first postnatal month of the mouse.

Cicero et al.[6] pointed out a neuronal localization of the 14-3-2 protein by finding a decrease in the content of the protein during nerve degeneration and an increase in the amount during nerve regeneration. Grasso et al.[3] also suggested a synaptosomal localization of the protein by biochemical methods. Due to these results we found it interesting to study the distribution of 14-3-2 by immunoelectron microscopy, and in this paper we have focused our attention on the synaptic region.

Material and methods. The 14-3-2 protein was purified from beef-brain according to Grasso et al.[2, 7], and its antiserum was prepared according to Cicero et al.[6]. The homogeneity of the antigen was controlled by SDS gel electrophoresis (15% polyacrylamide) according to