SOME SHORTCOMINGS OF DIRECT INTRAVENTRICULAR INJECTION IN MICE

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The use of X-ray photography following injection of iophendylate to verify that intraventricular injections in mice have been satisfactorily accomplished is misleading since it fails to detect leakage into the periphery. Radioactive labelling reveals that a substantial proportion of the injected material is rapidly carried to the periphery in the bloodstream.

Introduction Injections into the cerebral ventricles of mice can be made directly through the skull of the conscious animal (Haley & McCormick, 1957; Brittain, 1966). The process is quick, it produces surprisingly little trauma and it is convenient in that no prior surgery is required. The traditional method of verifying that the injection site is correct is to inject diluted Indian ink and subject the brain to histological examination. In more recent times injection of iophendylate followed by X-ray photography has been used. However, in the course of an investigation of polyamine synthesis and metabolism in mouse brain it was found that the recovery of radioactive material injected by the intraventricular route was much lower than iophendylate injection would seem to suggest. The experiments described here were carried out in order to substantiate and explain this discrepancy.

Methods Swiss-S albino mice weighing 20-25 g were injected by the intraventricular route with 20 μl iophendylate solution (Myodil, Glaxo) or with 20 μl of a solution of 1 μCi putrescine [1,4-^{14}C] dihydrochloride, sp. act. 63 mCi/mmol (Radiochemical Centre, Amersham), 1 μCi aminopropyl-tetramethylene [1,4-^{14}C] diamine (spermidine) trihydrochloride sp. act. 8.3 mCi/mmol (New England Nuclear), or 2.5 μCi S-adenosyl-(L-methionine-[2^{3}H]) sp. act. 2.0 Ci/mmol (Radiochemical Centre, Amersham) in 0.9% w/v NaCl solution. The injection was made on the left side at a point 1 mm from the midline and 2 mm forward of the anterior base of the ears. Location of the correct site is facilitated by the skull in the elective area being thinner than usual. A Hamilton microsyringe was used to deliver the injection through a short bevel 27 gauge needle which was fitted with a polythene tube stop that limited penetration of the skull to a depth of 3.2 mm. The target site was the left lateral ventricle. The animals were anaesthetized with ether immediately after injection and then subjected to X-ray photography or killed by exsanguination. Tissue samples weighing up to 400 mg or 0.2 ml blood were solubilized in 1.6 ml 1 M hyamine hydroxide in methanol by heating overnight at 60° C. The solution was then acidified with glacial acetic acid, decolourized with 30% hydrogen peroxide and mixed with 10 ml of a scintillant containing 0.5% w/v PPO (2,5-diphenyl-oxazole) and 0.01% w/v dimethyl POPOP (1,4-di-(2(5-phenyloxazole))-benzene) in two parts of toluene to one of Triton X-100. Radioactivity was determined in a Tracerlab liquid scintillation spectrometer using automatic external standard channels ratio quench correction.

Mouse blood volume was determined in five animals by isotope dilution. An intravenous injection of 100 μl ^{131}I-labelled human serum albumin (7 μCi/ml) was given via a tail vein and after 10 min the animal was anaesthetized with ether and exsanguinated. The radioactivity of a 100 μl sample of blood and of the injection solution was determined in a Packard 3375 spectrometer.

Results X-ray photography of mice injected with iophendylate confirmed that the injected material does enter the ventricular system. Figure 1 shows that 1-2 min after the injection the radio-opaque solution was present in both the left and the right lateral ventricles and the third and fourth ventricles and had also entered the cisterna. Careful examination of the photograph taken from the lateral aspect reveals some leakage of iophendylate into the subcutaneous tissue overlying the injection site.

However, the radioactive substances were all recovered in low yields from brain. The percentage recovery of putrescine was 21.7 ± 5.4, that of S-adenosylmethionine was 23.6 ± 4.7, and that of spermidine 17.0 ± 6.5. Within 2 min of the injection a substantial amount of the injected material was present in the blood of the animals. The blood volume of mice was found to be 89 ± 11 ml/kg, a figure which agrees with values found by previous investigators (Wish, Furth & Storey, 1950). Using

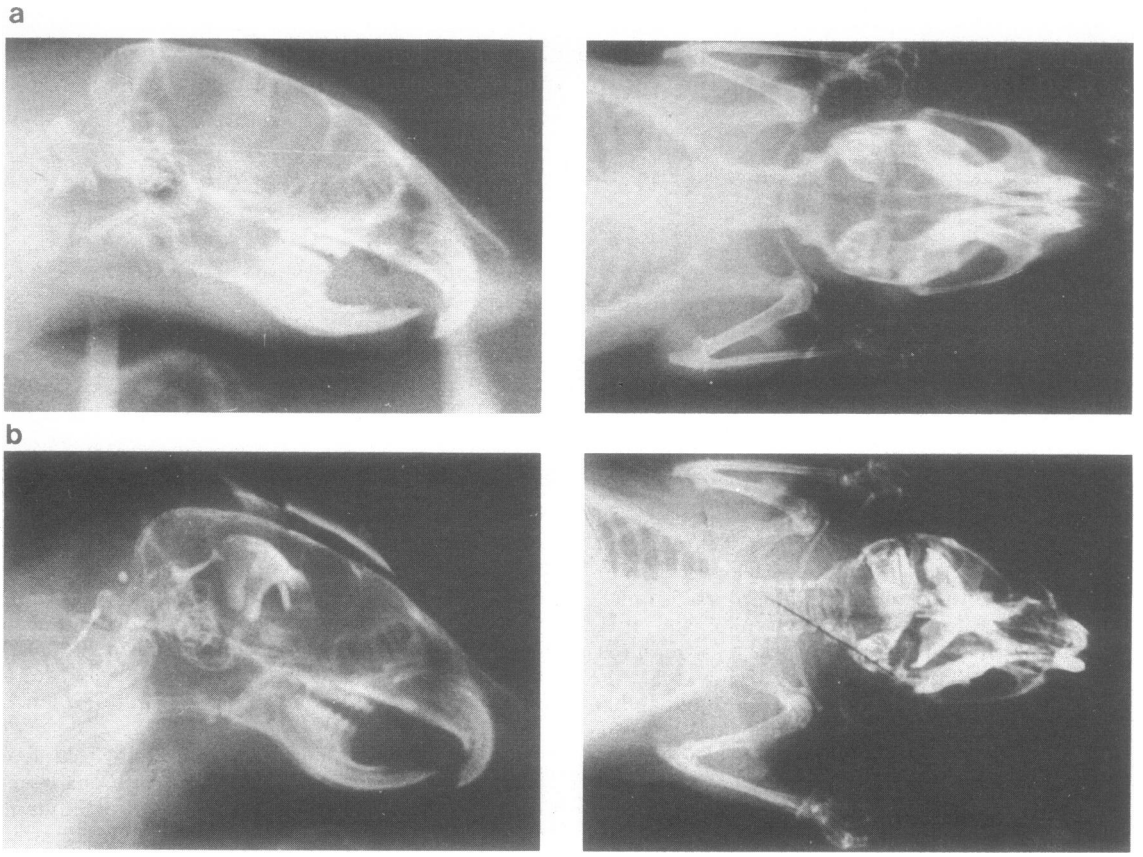

Fig. 1 X-ray photographs of mice taken from the lateral and dorsal aspects (a) before and (b) after an intraventricular injection of 20 μl iophendylate solution.

this value it was calculated that the percentage recovery of putrescine in blood was 21.4 ± 4.8. A further 10.1 ± 0.9% of the injected putrescine was recovered by washing the outer surface of the skull following removal of the brain. Only a very small proportion of putrescine was recovered in the liver (1.1 ± 0.5%).

When putrescine was injected in a volume of 10 μl the percentage recovered in brain was 22.9 ± 6.1. Reducing the injection volume to 5 μl increased the recovery to 34.6 ± 8.6%.

Discussion The experiments show that the use of X-ray contrast media to visualize the distribution of material injected into the cerebral ventricles can lead to a false sense of security. Although losses by leakage into the immediate area surrounding the injection site are detected, leakages into the periphery are not. The experiments using radioisotopes show that when this method of intraventricular injection in mice is used, leakage into the periphery is considerable. Metabolism of the injected material plays no part in their transfer to the periphery since the three substances injected were recovered in approximately the same proportion yet they are metabolized at widely different rates. The half-life of S-adenosylmethionine in mouse brain is 2.4 h, that of putrescine 6 h and that of spermidine 14 days. Numerous authors have previously reported that absorption into the blood stream takes place after intraventricular injection of histamine or adrenaline. This absorption has been attributed to circulation of the injected material into the subarachnoid space and its absorption by blood vessels or the arachnoid villi (Bedford, 1953; Bhawe, 1958; Draskoci, Feldberg, Fleischhauer & Haranath, 1960). The *pars tuberalis* has been suggested as a specific site for adrenaline absorption (Sproull, 1963). In general, however, absorption following injection

into the ventricles via indwelling cannulae is slow (DaSilva & Sproull, 1964) and only a minor proportion of the injected material reaches the blood stream. Cairns (1950) has shown that following the injection of 30 μl fluid into the subcortical tissue of mice, only 2-8% of the injected material is recovered in the brain. It has been suggested that the high injection pressure associated with injections made into tissue results in rupture of the arachnoid villi allowing passage of material into the sagittal sinus (Mims, 1960). It would seem likely that losses of material injected directly into the cerebral ventricles of mice could be explained in the same way since the injection volume traditionally used (20 μl) is large in relation to the volume of fluid likely to be present in the ventricular system of such a small animal and a major proportion of the injected material is rapidly absorbed. Cairns (1950) found that reducing the injection volume to 5 μl did not significantly reduce the overspill into the blood stream. In the present investigation recovery was unchanged when the injection volume was decreased to 10 μl but improved when 5 μl was injected. It would seem reasonable to suppose that with direct injection there would be a greater opportunity for the injection to leak back along the needle track than with an indwelling cannula implanted a week or more before injection. That such leakage occurs is clearly shown in the present investigation by the presence of injected material in the subcutaneous tissue overlying the skull. It is probably the route by which most of the misplaced material travels since it is the shortest route to the subarachnoid space.

The total recovery of putrescine in the present experiments when 20 μl was injected was 54%. The remaining putrescine was probably distributed throughout the peripheral tissues of the animal, as Janne (1967) has shown that the major proportion of putrescine injected by the intraperitoneal route is found in these tissues.

An obvious implication of these findings is that caution is needed in interpreting the effects of intraventricular injections made by this method since in some circumstances entry of the injected material into the periphery could be a complicating factor. At the same time it could be argued that substances such as catecholamines, 5-hydroxytryptamine and histamine which have been shown to change body temperature when injected by this route (Brittain & Handley, 1967; Shaw, 1971) are even more potent than was previously imagined.

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