For EM the perfused brains were also quickly excized and brain halves immersed in Karnovsky's paraform-glutaraldehyde solution. The samples were then washed overnight in phosphate buffer (pH 7.2) and postfixed for 2 h in 1% aqueous osmium tetroxide with 1.5% potassium ferrocyanide[10], dehydrated in ethanol and embedded in Spurr. Thin sections were stained with uranyl acetate and lead citrate and examined with a TESLA BS 500.

Results. a) Intraventricular application route. NMRI mice of both sexes tolerated the injected FITC-dextrans with no signs of distress, even over a period of several days. In all animals that recieved the FITC-dextran injection in the telencephalic ventricle, the ependyma and the plexus epithelium of the ventricles I–IV fluoresced intensely after 15 and 30 min. With the EM, the FITC-dextran could be seen as non-aggregated, relatively round electron dense particles. Independently of the route of administration, nearly 80% were stained darkly and evenly. Inside the ependyma cells, a number of particles were enclosed in double-walled vacuoles. The underlying glia cells, with their processes, had particles in vacuoles and independently scattered in the cytoplasm. Particles could be distinguished easily from the ribosomes. The labelled dextran particles could be found in the epithelium of the plexus chorioideus of all brain ventricles (figure 1). The opaque tracer seemed to have no tendency to bind to any particular cell organelles and could be distinguished from vacuoles and vesicles of various sizes. b) Intravenous application route. FITC-dextran injected i.v. could also be detected easily in the blood vessels and in the underlying tissue after the passage of the fenestrated capillaries (figure 2). The thrombocytes showed an uptake of tracer particles. 5 min after injection, the endothelium and the basement membrane in the plexus chorioideus were free of dextrans. The particles had already passed into the pericytes and the plexus epithelium. Some of the FITC-dextran particles had also passed the blood-brain-barrier in the cortex and were gathered in the cytoplasm of the astroglia.

Discussion. The results show that FITC-dextrans can be visualized for use not only in FM, but also as a useful tracer for studying the physiological, as well as the ultrastructural aspects of capillary permeability. This tracer is particularly attractive compared with other tracers such as ferritin[6] or peroxidase[7], because it is a non-toxic, stable polymer[13], detectable individually and directly; it is of biological origin and can be obtained commercially in a wide range of molecular weights.

In our tissue specimens there was no indication that the labelling of dextrans with fluorescein isothiocyanate interferes with staining. The present results, unlike those reported by Simionescu et al.[9] for rat intestinal tissue, showed undistorted, unaggregated tracer. The varying electron opaqueness of the tracer was not due to passage through the endothelium or basement membrane. These discrepancies could be due to different tracer molecular weights, fixation procedure, and/or staining method. Ainsworth[10] did not observe aggregations of dextrans (mol.wts 40,000–250,000) in the kidneys of mice and rats.

The transcapillary transport of FITC-dextran 150 in the brain took place in the plexus chorioideus. Analogous results have been found in the fenestrated capillaries of hamster and rat brain[12]. So far as FM data[11,12] are concerned a blood-brain-barrier appears to exist in the other vascular walls of the brain. However, our EM specimens of the brain cortex clearly show single particles in the astroglia shortly after intravenous application which may have escaped detection by FM.

FITC-dextrans seem to be attractive tracers both for FM and EM studies of permeability, to elucidate the correlation of physiological data with structural findings at the ultrastructural level.

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A simple method for the verification of a successful cannulation of the rat cerebral ventricles[1]

I. Paakkari

Department of Pharmacology, University of Helsinki, Siltavuorenpenger 10, SF-00170 Helsinki 17 (Finland), 27 August 1979

Summary. A simple and inexpensive method for the cannulation of the cerebral ventricles of the rat is described. The required tools consist of a stereotaxic instrument, a standard steel injection cannula and a polyethylene tube.

Intracerebroventricular administration of drugs is a commonly used method for studying the central nervous system. Usually the proper injection site is verified only after the experiment in the autopsy by leaving the cannula in situ or by injecting some dye such as methylene blue into the ventricle. However, in experiments which may last for several hours or even several days a considerable amount of work is spared if the correctness of the puncture is verified immediately after the insertion of the cannula, which enables unsuccessful punctures to be discarded at the very beginning of the experiment.

The applicability of stereotaxic coordinates to the ventricular puncture is lessened if the experimental animals differ in strain, sex or weight from those used for the derivation of the coordinates in the stereotaxic atlases. Furthermore, the brain topography may change considerably after the open-

ing of the dura due to the entry of air through the puncture hole[2]. In big animals such as cats or dogs the correct puncture usually results in spontaneous outflow of cerebrospinal fluid (CSF)[3-6]. However, in smaller animals such as rats one rarely obtains CSF in this manner. The use of an outer guide cannula and an internal injection cannula have been employed by some investigators to ascertain the proper cannula placement by the appearance of CSF[7]. Connection of the cannula system to a pressure transducer with a subsequent amplification and recording of the signal has been used to detect the higher ventricular pressure at the moment of the penetration of the ventricle by the cannula[8]. Another method employed a positive pressure in the injection cannula created by constant infusion of artificial CSF, whereby a fall of the pressure was measured with a transducer after the penetration of the ventricular wall[9]. Furthermore, even X-ray techniques have been used to ascertain the proper position of the cannula[4].

The present paper describes a simple method by which the proper position of the cannula in the cerebral ventricle can be verified without any other technical device than a stereotaxic instrument.

Rats weighing 250–300 g are anaesthetized with an i.p. injection of urethane (1.5 g/kg). A 26 gauge steel cannula with a length of 34 mm is attached to a 45 cm long polyethylene tube (PE-50), which is marked at 45 mm intervals corresponding to the volume of 10 µl. The cannulation system is filled with saline and the free end of the polyethylene tube is closed hermetically. To make a puncture into the lateral ventricle a hole is drilled 5.3 mm posterior to the bregma and 4 mm lateral to the sagittal suture. The cannula is lowered 1.9 mm from the dura surface. The corresponding coordinates in the case of the 4th ventricle were 10.2 mm posterior, 0 mm lateral and 6.2 mm vertical. To find out whether the needle is correctly positioned inside the ventricle the free end of polyethylene tube is opened and kept at the level of the skull surface. Occasionally one observes an upward movement of the saline meniscus in the tube signifying a successful puncture. However, in the majority of cases the closely adjacent brain tissue or the choroid plexus in the possibly collapsed ventricle[2] acts as a valve and hinders the outflow of CSF through the cannula. To clear the cannular lumen a positive hydrostatic pressure is created in the injection system by lifting the flexible polyethylene tube along a centimeter scale which is zeroed at the level of the needle tip. The height where the saline meniscus in the tube starts flowing downwards indicates the intraventricular pressure in cm saline plus the pressure needed to clear the possibly obstructed cannula tip. After the start of the influx the tube is immediately lowered till no saline flow is observed. From this point the tube is again slowly raised and the point at which the saline inflow starts is recorded (figure 1). No more than 3–5 µl of saline escapes into the ventricle during the pressure measurement. If no inflow of saline is noted at the hydrostatic pressure of 25 cm saline the cannula is moved upwards by 0.1 mm and the procedure is repeated. The mean CSF pressure in the lateral ventricles of 126 rats was 13.2 ± 3.3 (SD) cm of saline (figure 2).

For drug injections the polyethylene tube is removed from the needle and filled with the solution to be infused and then again attached tightly to the needle. If great exactness is required the dead space of the needle, 1.8 µl, can be taken into consideration when the dose of the effective drug is calculated.

The final approval of the experiments is made afterwards when the autopsy of the rats has shown that the injection cannula was situated correctly in the ventricle. In a series of 126 injections into the lateral ventricle the cannula placement was correct in all but one case.

The highest hydrostatic pressure used in this method is the same as the lowest pressure used in the previously described insertion technique[9]. In fact, on most occasions the saline inflow starts at a pressure level which only marginally exceeds the ventricular pressure. The small positive pressure minimizes the likelihood of tissue injections and

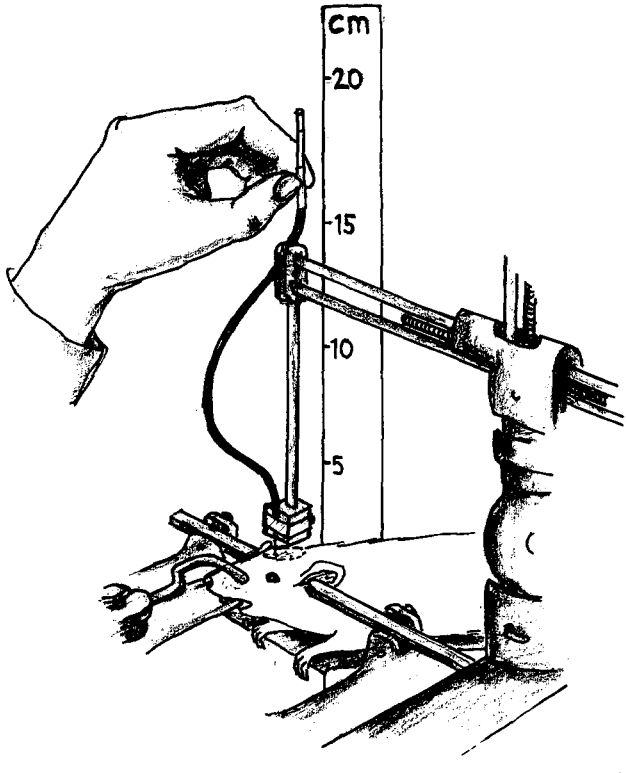

Fig. 1. Measuring of the intraventricular pressure of the rat cerebral ventricle. A flexible polyethylene tube filled with saline is attached to the ventricular cannula. Hydrostatic pressure in the cannula is increased by lifting the tube slowly upwards along a centimeter scale which is zeroed at the level of the cannula tip. The height of the saline column sufficient to start an inflow of saline is noted as the intraventricular pressure in cm of saline.

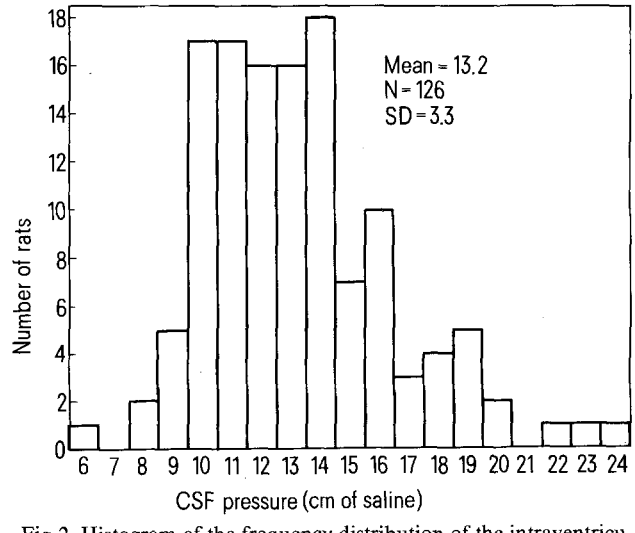

Fig. 2. Histogram of the frequency distribution of the intraventricular (lateral) pressures of 126 male Wistar rats weighing 250–300 g. The measurement was made in urethane anaesthesia (1.5 g/kg).

the damage of the periventricular structures. An additional advantage of this method is the simplicity and unexpensiveness of the technical tools as compared to the methods using pressure transducers and recording devices[8,9].

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Immunogenicity of agarose-immobilized immune complexes[1]

P.J. Higgins

Laboratory of Cell Biochemistry, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York (N.Y. 10021, USA), 8 October 1979

Summary. A novel method is described for production of heterologous antisera to a specific tumor-associated murine antigen by immunization with agarose-trapped immune complexes.

Preparation of high titer heterologous antisera to specific tumor-associated antigens is complicated by the use of crude tissue extracts as immunogen in which the relative concentration of the antigen is quite low. Where the tumor-associated antigen is present in moderate quantities, reasonable quality antisera can be obtained by immunization with unfractionated tissue extracts and appropriate serum absorptions. Such antisera have been used to precipitate antigen from solution and the immune complexes, in turn, employed as immunogens to elicit the formation of a more highly specific antiserum[2]. The limiting factors, however, of sufficiently absorbed precipitating antibody, low concentrations of specific antigen in crude tissue extracts and difficulties in the efficient processing of minute precipitates combine to make effective immunization with solution-precipitated immune complexes a prodigious task.

A simple method for the preparation of high titer antisera to a specific tumor-associated antigen, murine γ-FA[3-5], is described. This procedure should be applicable to studies of diverse tissue antigens.

Methods and results. Immunization of rabbits with saline extracts of a mouse fibrosarcoma, antiserum absorption and subsequent identification of an antigen common to tumor, fetal and adult splenic tissue, termed γ-FA, has been described[3-5]. Radial immunodiffusion plates[6] contained 0.1 ml of absorbed anti-γ-FA serum and 2.5 ml of 1% agarose (w/v) in Beckman B-2 buffer, pH 8.6. Antigen wells (3.7 mm in diameter) were cut into the agarose gel, filled with 7 µl of the 10,000 × g supernatant fraction of a 10^{-3} M Tris, pH 7.5, homogenate of normal adult mouse spleen and the plates incubated at 37 °C for 72 h to allow for precipitin ring formation. The agarose slabs were then dialyzed with stirring against daily 200-ml changes of phosphate-buffered saline (PBS) for 3 weeks at 4 °C in order to remove unbound protein. The use of a 1% agarose gel facilitated this removal while antigen-antibody complexes remained trapped within the gel matrix. After dialysis, sections of gel containing precipitin rings were cut out, passed several times through an 18-gauge needle and frozen at −20 °C in twice the volume of PBS. On day 1, 14 and 21, 1 ml of agarose-immobilized immune complexes was emulsified in 1 ml of complete Freund's adjuvant and inoculated s.c. into a New Zealand white rabbit (on day 21 incomplete adjuvant was substituted for complete adjuvant). 22 days later the rabbit was bled and 1-ml aliquots of the antiserum inoculated i.p. into each of several adult C57 mice for in vivo absorption. After 24 h, the mice were bled and the antibody activity of the absorbed antiserum compared with that of the original anti-γ-FA serum. Clearly, the antiserum to γ-FA-anti-γ-FA immune complexes possessed all the precipitin specificity of the original anti-γ-FA serum but at a much higher titer (table) and, unlike the original antiserum, yielded a positive indirect immunofluorescence test[4] on methanol-fixed rat hepatoma cells (figure). This antigen was previously thought to be synthesized only by in vivo propagated tumor cells[4] and the present data, therefore, provide the 1st direct evidence for production of γ-FA by transformed cells. Moreover, reten-

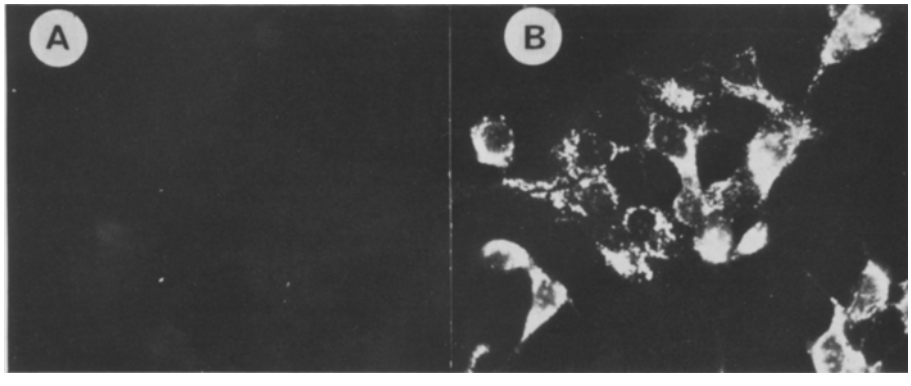

Fig. 1. Indirect immunofluorescence test of in vitro propagated rat tumor cells isolated from a transplanted hepatoma[7]. *A* Original anti-γ-FA serum (1:20); *B* anti-γ-FA immune complexes (1:60). UV light microscopy, BG12-53/44 filters, Zeiss Photoscope.