death. The commonly used parenteral antiepileptic drugs appear to be effective in raising the threshold, both phenobarbitone and phenytoin affording protection against seizures at doses producing no obvious behavioural change, while diazepam was effective only at doses producing behavioural toxicity (somnolence, ataxia). Since, of the drugs tested, phenobarbitone was effective at lower doses and had a greater maximal elevating effect on the aminophylline seizure threshold, it would appear to be the agent of choice in the rat model. Whether it would be effective in preventing seizures in patients treated with parenteral aminophylline remains to be resolved.

REFERENCES

Dunnet, C. S. (1964) Biometrics 20: 482–491
Man, D., Comsroe, P. F. (1973) Life Sci. 12: 429–434
Schwartz, M. S., Scott, D. F. (1974) Epilepsia 15: 501–505
Yarnell, P. R., Chu, N. S. (1975) Neurology 9: 819–822
Zwillich, C. W., Sutton, F. D., Neff, T. A. (1975) Ann. Int. Med. 82: 784–787

J. Pharm. Pharmacol. 1981, 33: 480–482
Communicated February 3, 1981

0022-3573/81/070480-03 $02.50/0

Intrathecal injections in rats by percutaneous lumbar puncture

G. H. SLOANE-STANLEY*, ROSEMARY A. CHASE, *Department of Biochemistry, Institute of Basic Medical Sciences, Royal College of Surgeons of England, 35/43 Lincoln's Inn Fields, London WC2A 3PN, U.K.*

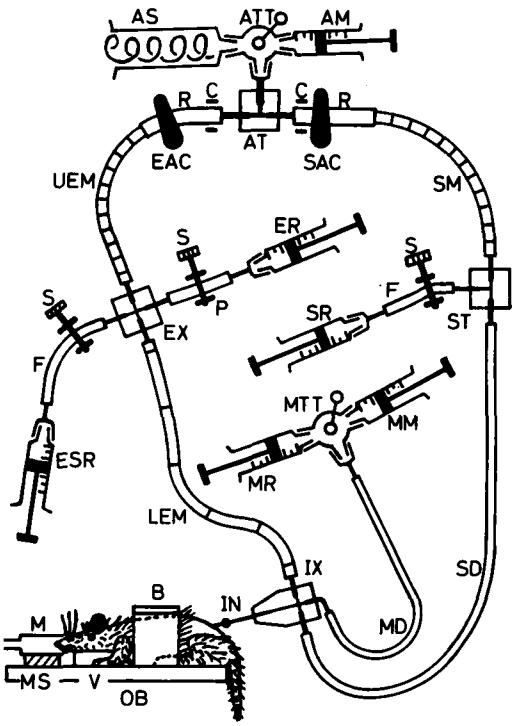
Drugs can often best be delivered to the central nervous system intrathecally, the intracisternal or intraventricular routes usually being the most accessible in small animals (e.g. Jeffers & Griffith 1949; Noble et al 1967). However, for some quantitative experiments on tetanus, we preferred to make lumbar punctures, because they *are used more commonly in man*, incur no risk of mechanical damage to the brain or brain stem, and need neither preparative surgery nor stereotaxic apparatus. A procedure for making and monitoring such injections in rats, the smallest animals likely to be technically suitable, is now described.

Male rats of an inbred Norwegian strain, 200–230 g, were first given an injection of about 8 LD50 of tetanus toxin in 100 mm^3 of gelatin-phosphate buffer into the right gastrocnemius muscle. The animals were then anaesthetized and an injection of tetanus antitoxin made into the lumbar cerebrospinal fluid (c.s.f.) either before the onset of tetanus, for prophylaxis, or after the first signs had appeared. This was followed by X-ray contrast oil via the same needle to permit radiographic monitoring. Rats in which tetanus progressed beyond a mild and apparently painless stage were killed by anaesthesia.

For lumbar punctures and radiography, the animals were anaesthetized with halothane in oxygen from an open-circuit apparatus similar in principle to that of Reese & Nunn (1961). The mixture was partially humidified by passage over water. Induction was rapid with 5% (v/v) halothane in oxygen at 3·25 l min^{-1}; anaesthesia was then maintained by 1–3% halothane in oxygen. The animal was placed prone on an operating-board carrying a fixed V-shaped support for the mandible and a loose bridge for the operator's hand to prevent compression of trachea and lungs (Fig. 1).

The injection was then made from an apparatus based on that of Jeffers & Griffith (1949) for intracisternal injections from a narrow graduated tube under known low pressures (Fig. 1). The injecting needle (IN of Fig. 1) was a double-ended cartridge-type Solila dental needle, 26 s.w.g. (0·45 mm) diam. and 1″ or 1¼″ (25 or 32 mm) from tip of long end to ball, and with a Hüber point on its long end (Amalgamated Dental Trade Distributors Ltd., London). The tubing for liquids was mostly polyethylene (Portex Ltd., Hythe, Kent), which could be permanently graduated with waterproof ink and which remained flexible, transparent and hydrophobic after repeated use, but 'Flexible Blue' (Portex) nylon tubing was used for MD of Fig. 1, because of its greater flexibility and oil- and pressure-resistance, while F.E.P. (Teflon: Dupont Ltd., Reading. Berks.) was used where neither graduation nor great flexibility were needed, because it was more durable and hydrophobic. The polytrimethylpentene (TPX: ICI Ltd., Welwyn Garden City, Herts.) 'T'- and 'X'-junction pieces shown in Fig. 1 were fabricated by drilling polished blanks and fitting them with stainless steel tube connections made from hypodermic needles. They were also used, together with clips on the plastic tubing, as 2- or 3-way stopcocks in situations where conventional stopcocks leaked unpredictably and/or had too much dead space; commercially available stopcocks were suitable for ATT and MTT of Fig. 1. The reservoirs for saline (NaCl, 154 mM), and for the dilutions of tetanus antitoxin in saline, were mostly sterile disposable polystyrene syringes, but polypropylene and glass ones were used both for storing and dispensing X-ray contrast oil (Myodil: Glaxo Ltd., Greenford, Middlesex) which attacks polystyrene, and when resterilization was desirable. They were connected to tubing via shortened and blunted hypodermic needles with nylon hubs. The apparatus was mounted in laboratory clamps and adjusted to exclude strain on the needle IN once inserted into the canal. Calibration was checked by making dummy injections of rat serum (followed by X-ray contrast oil: see below) into known volumes of saline; the E_{280} was then measured spectrophoto-

* Correspondence.



Item	Function	Material(s)	Size etc.
'P.P.40'	Flex. tubing	Polyethylene	1·5 mm o.d. × 0·5 mm i.d., fitted to 0·5 mm needle tubing
'Flexible Blue'	,, ,,	Nylon	0·75 mm o.d. × 0·5 mm i.d., fitted to 0·5 mm needle tubing
'F.E.P.28'	,, ,,	F.E.P.	1 mm o.d. × 0·5 mm i.d., fitted to 0·65 mm needle tubing
V	Mandible support	Varnished wood	Opening is 50 × 22 × 8 mm
OB	Operating board	,, ,,	Minimum 27 × 15 cm
B	Bridge	,, ,,	About 18 × 8 × 5 cm (handed for operator)
P	Conn. for liquids	P.P.40	Up to 10 cm long
F	,, ,,	F.E.P.28	,, ,, ,, ,,
M	'Mask'' ,,	Plastic	14 mm i.d.
MS	Mask support	Varnished wood	70 × 50 × 8 mm
R	Conn. for air	Silicone rubber	1·75 mm o.d. × 0·75 mm i.d. × 25 mm long, fitted to P.P.40 and to 1·08 mm needle tubing
C	Clamping collar	Rigid nylon tubing	3·2 mm o.d. × 1·8 mm i.d.
S	Screw clip	Plated brass	Smallest available, jaw edges rounded
AS (optional)	Air sterilizer	Cotton wool in polypropylene	50 cm³ syringe barrel
ATT (optional)	3-way air tap	Polypropylene	2 mm i.d. Luer connections
AM	Air measurer	Polystyrene	1 cm³ 'tuberculin'
AT	Air T-piece	T.P.X.	20 × 15 × 5 mm, holes drilled 0·97 mm for 1·08 mm needle tubing
EAC	'Experimental' air-clip	Stainless steel artery forceps for quick action	37 mm, jaw edges rounded
SAC	'Saline' air-clip		
UEM	Upper 'exper. fluid' measure	Graduated P.P.40	About 58 cm long, 100 mm³ capacity
SM	Saline measure	Graduated P.P.40	About 65 cm long, 110 mm³ capacity
ER	'Exper. fluid' reservoir	Polystyrene	As required
ESR	'Exper.' side's saline reservoir	,,	At least 10 cm³
SR	Saline reservoir	Polypropylene or Polystyrene	50 cm³ 10 cm³
EX	Exper. X-piece	T.P.X.	20 × 20 × 3 mm, holes drilled 0·43 mm, openings then enlarged to 0·475 or 0·58 mm for 0·5 or 0·65 mm needle tubing
ST	Saline T-piece		
LEM	Lower 'exper. fluid' measure	Graduated P.P.40	About 28 cm long, 50 mm³ capacity
SD	Saline delivery tube	F.E.P.28	25–30 cm long, about 50 mm³ capacity
MD	'Myodil' delivery tube	'Flexible blue'	About 40 cm long, 80 mm³ capacity
MTT	3-way 'Myodil' tap	Polypropylene	2 mm i.d. Luer connections
MM	'Myodil' measure	Glass and stainless steel	100 mm³
MR	'Myodil' reservoir	Polypropylene	5 cm³
IX	Injection X-piece (Actually perp. to plane of paper)	T.P.X.	30 × 18 × 3 mm, holes drilled 0·43 mm, then upper three openings enlarged as in EX and ST
IN	Injection needle	Stainless steel	See text

FIG. 1. Rat lumbar injection apparatus (not to scale)

metrically and indicated recoveries of 97·5, s.d. 2·5% (n = 5). Before use, the apparatus (without its reservoirs) was immersed in boiling water for 35 min before filling with sterile fluids. The lower experimental measure LEM and injection piece IX were flushed out with saline and IX was boiled and a new sterile needle (IN of Fig. 1) fitted (short end up). Immediately before each injection the rat lumbosacrum was shaved and it and the operator's hands were swabbed with surgical spirit.

To insert the needle IN, a right-handed operator gripped the ilium firmly with the left hand, fingers pointing caudally, then with the right hand he located the neural spine of S2, and, with an assistant maintaining an air pressure of about 3 kPa on the saline side of the injection apparatus (EAC closed: Fig. 1), he took the injection piece IX (Fig. 1) and pushed the needle vertically through the skin just caudal and lateral to S2 until halted by the sacrum. The ilium was then lifted to arch the back, and the point of the needle gradually advanced rostrally along the fused lateral zygapophyses until it entered the vertebral canal through the gap between the neural arches of L6 and S1. Penetration of the canal was accompanied by a sudden decrease in resistance to forward movement of the needle and by outflow of saline, whereupon the needle was passed

forward within the canal almost as far as its length allowed, the ilium being allowed to drop a little for the last 5 mm or so of the forward movement to avoid tearing the *dorsal* theca, to close the gap between L6 and S1 and to keep the needle firmly in position. The needle was then withdrawn slightly and if it was insecure or at an unusually steep or shallow inclination to the vertical, it was not in the vertebral canal.

Once lumbar puncture had been achieved, the air was switched to the experimental side of the apparatus (Fig. 1), and at about 7 k Pa, 60 mm^3 of antitoxin was passed into the canal at about 200 $mm^3\ h^{-1}$, i.e. 1/50 of goats' CSF-flux (brain wt 100 g: Heisey et al 1962). Both air inlets (EAC and SAC of Fig. 1) were then closed, the fluid levels noted, and 30 mm^3 of X-ray contrast oil injected from MM (Fig. 1) at an unknown pressure, after which the fluids were allowed to fall to their previous levels. The needle was then cut through behind the ball and thus sealed, and the rat removed in a plastic box containing a suitable halothane-oxygen mixture, for X-ray with the needle still in place; lateral and then dorsal views were obtained from a vertical beam (Fig. 2). The needle was then withdrawn, and the animal allowed to recover from the anaesthetic; this stage was reached about 45 min after induction of anaesthesia. A typical X-ray following a successful injection is reproduced in Fig. 2 (it may be seen that the distribution of the oil within the vertebral canal changed significantly between L1 and L2, suggesting this to be the level of termination of the spinal cord, so that needle-damage, if any, was probably limited to the nerves of the *cauda equina*). With practice, the proportion of failures became very small; the antitoxin was occasionally *more* (but never *less*) effective than the X-rays suggested. Some leaks appeared to be caused by—in order of increasing importance—the initial insertion of the needle being too lateral or too caudal to the second sacral vertebra, the use of a needle with an over- or under-bent Hüber point, and/or the operator's insensitivity to resistance of movement of the needle along the vertebral canal once it had been inserted between L6 and S1. Leaks may therefore have been caused by local destruction of the theca caught between the point of the needle and the wall of the vertebral canal.

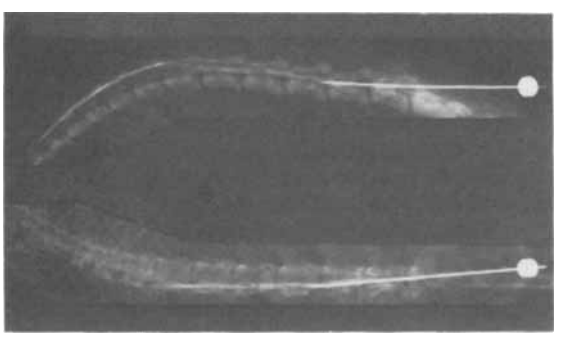


FIG. 2. X-radiographs, taken under anaesthesia, of part of rat's vertebral column after injection of tetanus antitoxin, followed by 30 mm^3 of contrast oil, through needle on right. Head to left. Lateral view above, dorsal view below. Needle length, tip to ball, 32 mm.

The safety and effectiveness of the procedure were shown by the absence of visible ill-effects on recovered animals, absence of signs of infection or injury at *post mortem*, and by the observation that in prophylactic experiments, rats given sufficient intrathecal antitoxin *did not develop signs of tetanus* or other abnormality. It appears, therefore, that the sterile precautions were adequate, that no significant mechanical damage was done to the nervous system, and that the injections were therapeutically efficient. These conclusions may merely reflect the general toughness of *R. norvegicus*, but they do seem to vindicate the use of the percutaneous lumbar route for intrathecal injections in that species as a model for similar injections in man.

We are deeply indebted to Professor C. Long, F.R.S.E., lately Head of this Department, and to Dr W. E. van Heyningen for constant encouragement and support; to the Wellcome Foundation for a personal grant to R.C. and for defraying the special expenses of this work, and to Dr André Turpin for a generous gift of pure tetanus toxin. We are also most grateful to Professor D. Slome, lately Head of the Department of Physiology of this Institute, for the use of X-ray apparatus and a dark-room, and to Dr H. W. Reading and Dr J. W. D. Bull, C.B.E., for essential advice on anaesthesia and radiography.

REFERENCES

Heisey, S. R., Held, D., Pappenheimer, J. R. (1962) Am. J. Physiol. 203: 775–781

Jeffers, W. A., Griffith, J. Q. (1949) in: Griffith, J. Q., Farris, E. J. (eds) The Rat in Laboratory Investigation. 2nd edn, Lippincott, Philadelphia, pp 196–197

Noble, E. P., Wurtman, R. J., Axelrod, J. (1967) Life Sci. 6: 281–291

Reese, A. J. M., Nunn, J. F. (1961) Br. J. Anaesth. 33: 54–57