wide variety of plant species in genera including *Senecio, Crotalaria, Heliotropium, Trichodesma, Amsinckia*, and others. Within these species at least 100 different pyrrolizidine alkaloids have been identified and their structures elucidated (*2*). Livestock poisoning by consumption of *S. jacobaea* and other pyrrolizidine alkaloid–containing plants is a major problem in Oregon (*17*) and other parts of the United States and the world (*18*). The transmission of these alkaloids or their metabolites to the consumer through meat and dairy products from exposed animals must also be considered possible.

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References and Notes

1. *Senecio jacobaea* was apparently introduced to North America through the port of Pictou, Nova Scotia, from Scotland in ship ballast around the 1850's [J. M. Greenman, *Ann. Mo. Bot. Gard.* **2**, 602 (1915); W. H. Pethick, *A Special Report on Pictou Cattle Disease, 1906* (Canada Department of Agriculture, Health of Animals Branch, Government Printing Bureau, Ottawa, 1907)]. A local livestock disease called Pictou disease results from ingestion of *S. jacobaea* [see L. B. Bull *et al.* (*2*)]. The first known record of tansy ragwort from the Pacific Northwest is from Nanaimo, British Columbia, in 1913, and the plant was first discovered in Oregon in 1922 in a ballast dump in Portland, Oregon [D. L. Issacson, thesis, Oregon State University (1973), p. 65].
2. L. B. Bull, C. C. J. Culvenor, A. T. Dick, in *The Pyrrolizidine Alkaloids* (North-Holland, Amsterdam, 1968).
3. R. B. Bradbury and C. C. J. Culvenor, *Aust. J. Chem.* **1**, 378 (1954).
4. A. R. Mattocks, in *Phytochemical Ecology: Proceedings*, J. B. Harborne, Ed. (Academic Press, London, 1972), p. 179.
5. E. K. McLean, *Pharmacol. Rev.* **33**, 429 (1970).
6. D. J. Svoboda and J. K. Reddy, *Cancer Res.* **32**, 908 (1972); P. N. Harris and K. K. Chen, *ibid.* **30**, 2881 (1970); J. R. Allen, I. C. Hsu, L. A. Carstens, *ibid.* **35**, 997 (1975).
7. A. M. Clark, *Nature (London)* **183**, 731 (1959).
8. C. R. Green and G. S. Christie, *Br. J. Exp. Pathol.* **42**, 369 (1961).
9. In ragwort-endemic areas of the Pacific Northwest, knowledgeable beekeepers recognize the presence of ragwort honey through the appearance of excessive yellowing (travel staining) of the wax combs and internal hive structures (wooden frames). This is caused by the bees "tracking" ragwort pollen over the surfaces of the combs.
10. J. Louveaux, A. Maurizio, G. Vorwohl, *Bee World* **51**, 125 (1970). The pollen grains of *S. jacobaea* are typically ovoid and extremely spinous, characteristic of pollens from the Compositae family. Individual pollen grains are 25 to 30 μm in diameter.
11. A. R. Mattocks, *Anal. Chem.* **39**, 443 (1967).
12. J. B. Bingley, *ibid.* **40**, 1166 (1968).
13. N. Neuner-Jehle, H. Nesvadva, G. Spiteller, *Monatsh. Chem.* **96**, 321 (1965); T. Furuya and K. Arake, *Chem. Pharm. Bull.* **16**, 2512 (1968).
14. D. N. Patwardhan and J. W. White, Jr., in *Toxicants Occurring Naturally in Foods* (National Academy of Sciences, Washington, D.C., ed. 2, 1973), p. 495.
15. H. B. Wood, Jr., V. L. Stromberg, J. C. Kereszтesy, E. C. Horning, *J. Am. Chem. Soc.* **76**, 5689 (1954).
16. E. Crane, in *Honey, A Comprehensive Survey* (Heinmann, London, 1975), p. 125.
17. D. L. Isaacson, *Proceedings of the 24th Oregon Weed Conference*, 21–23 October 1975 (Pacific Northwest Regional Commission, Vancouver, Wash., 1975), vol. 1, pp. 1–3; S. P. Snyder, *Oreg. Agric. Res.* **225**, 2 (1972).
18. O. H. Muth, *J. Am. Vet. Med. Assoc.* **153**, 310 (1968).
19. We thank the National Institute of Environmental Health Sciences (grant ES 00210) and the Pacific Northwest Regional Commission (grant AG-3004) for support of this work. This is Technical Paper No. 4300 from the Oregon Agricultural Experiment Station, Oregon State University, Corvallis.

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Primate Model for Long-Term Study of Intraventricularly or Intrathecally Administered Drugs and Intracranial Pressure

Abstract. *Meaningful pharmacokinetic investigations require animal systems which approximate the human situation. This report describes a primate model in which silicone catheters are placed into the fourth ventricle and the spinal subarachnoid space and connected to subcutaneous cerebrospinal fluid reservoirs. This model permits sterile access to ventricular cerebrospinal fluid without tissue damage, provides mixing of injected drugs with lateral ventricular cerebrospinal fluid, enables spinoventricular perfusion, and permits ventricular cerebrospinal fluid sampling over extended periods in unanesthetized rhesus monkeys. This animal system may provide intraventricular pressure recordings and pharmacokinetic data similar to that obtained in man.*

Rational approaches to the treatment of central nervous system malignancies demand precise knowledge of the pharmacokinetic behavior of chemotherapeutic agents in cerebrospinal fluid (CSF) (*1, 2*). Meaningful pharmacokinetic investigations require animal systems which approximate the human situation. Unfortunately, currently available animal models have been plagued by technical problems and have not provided long-term access to ventricular CSF with any reliability.

Most model systems require immobilization of the animal for long periods or anesthesia, and passage of cannulas through the cerebrum. These techniques do not provide experimental physiologic conditions and are associated with tissue damage (*3, 4*), infection, and catheter obstruction (*5*). Moreover, repetitive cisternal or lumbar punctures yield fluid that only simulates ventricular CSF and do not promote mixing of injected drugs with ventricular CSF.

The recently expressed need for suitable animal models (*4, 6*) prompted our development of a primate system which avoids these shortcomings. This model is based on an adaptation of the subcutaneous Ommaya CSF reservoir (*7*) developed for the treatment of central nervous system neoplasms in man (*1*). Preliminary physiologic and pharmacokinetic evaluations with this animal system demonstrate a close correlation with similar investigations in man.

Rhesus monkeys were anesthetized during the surgical implantations. A midline posterior incision was made over the occipitocervical junction and the posterior margin of the foramen magnum was removed (*8*). After midline dural incision, the foramen of Magendie was dilated to enable the insertion into the fourth ventricle of a silicone Pudenz catheter (2 mm in outside diameter). Each catheter tip was placed no less than 3 mm from the exit of the aqueduct of Sylvius to prevent aqueductal obstruction. After the dura was closed and made watertight with fine silk sutures, a 2.5-cm side-armed Ommaya CSF reservoir was connected to the catheter and secured subcutaneously over the occipital bone (Fig. 1, left). Intraoperative magnification is not necessary for the performance of this procedure.

Immobilization of the animal in a chair permitted access to the reservoir without anesthesia. The physiologic characteristics of our animal model were documented by the injection of isotopically labeled serum albumin into the ventricle, by positive contrast ventriculography, and by intracranial pressure recordings (*8*). At the conclusion of each evaluation period, the animals were returned to their cages and thus did not require long-term immobilization.

That the CSF of the fourth ventricle mixed with that of the lateral ventricle and flowed against the natural circulation pattern was verified by isotope injection and positive contrast ventriculography. After the injection of technetium 99m–labeled serum albumin, radioactivity migrated from the reservoir into the fourth ventricle and cisterna magna immediate-

ly after the reservoir was depressed manually two times (Fig. 2); the radioactivity mixed throughout the ventricular system after four such pumpings. Similarly, positive contrast material injected into the reservoir could be detected in the lateral ventricle after four pumping maneuvers. Ventriculograms obtained in this manner were similar to those previously reported in rhesus monkeys (*9*) and man (*10*).

A scalp vein needle (23 gauge) was inserted percutaneously into the Ommaya CSF reservoir and connected to a pressure transducer (*11*) (Fig. 2). Intraventricular pressure tracings fluctuated appropriately with arterial pulsation, respiratory activity, and bilateral compression of the jugular vein, and were similar to those obtained from man (*12*).

Results of the initial application of our primate model to the pharmocokinetic investigation of methotrexate injected into the CSF are presented in Fig. 3. Molar concentrations of methotrexate in the CSF were determined by a dihydrofolate reductase (E.C. 1.5.1.4) inhibition assay (*13*). The disappearance curves for intraventricularly injected methotrexate in our animals were compared with those determined in 93 patients with similar subcutaneous reservoirs. The curves for our monkeys are almost parallel and the absolute concentrations are proportional to dosage. Intrareservoir injections of methotrexate (15 mg per square meter of animal surface area) in our monkeys yielded CSF concentrations which most closely approximate those noted in patients receiving 12 mg/m² methotrexate by way of Ommaya reservoirs.

Our model may be adapted to enable spinoventricular perfusions by installing a T-shaped Hoffman catheter in the spinal subarachnoid space (*14*) (Fig. 1, right). In this system, communication between the spinal and fourth ventricular catheters was demonstrated by positive contrast myeloventriculography.

That the catheter remained open and sterile over long periods of time was verified by the biweekly withdrawal of 2 ml of CSF for bacterial cultures for 6 months. When the animals were killed, we found no catheter or ventricular obstructions and no microscopic lesions.

We conclude that spinal and fourth ventricular catheterization with the use of subcutaneous Ommaya reservoirs in rhesus monkeys (i) permits sterile access to CSF without needle tract artifacts or long-term immobilization, (ii) provides mixing of injected drugs with lateral ventricular CSF, (iii) enables spinoventricular perfusion, (iv) permits ventricular CSF sampling over extended periods, (v) enables sensitive monitoring of intra-

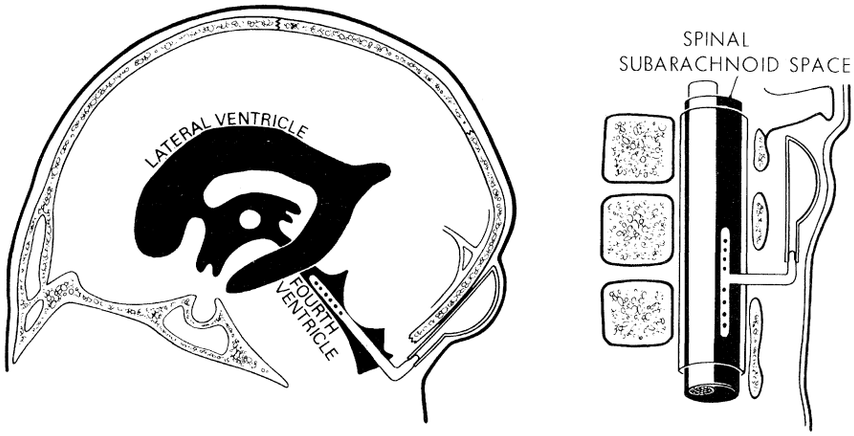
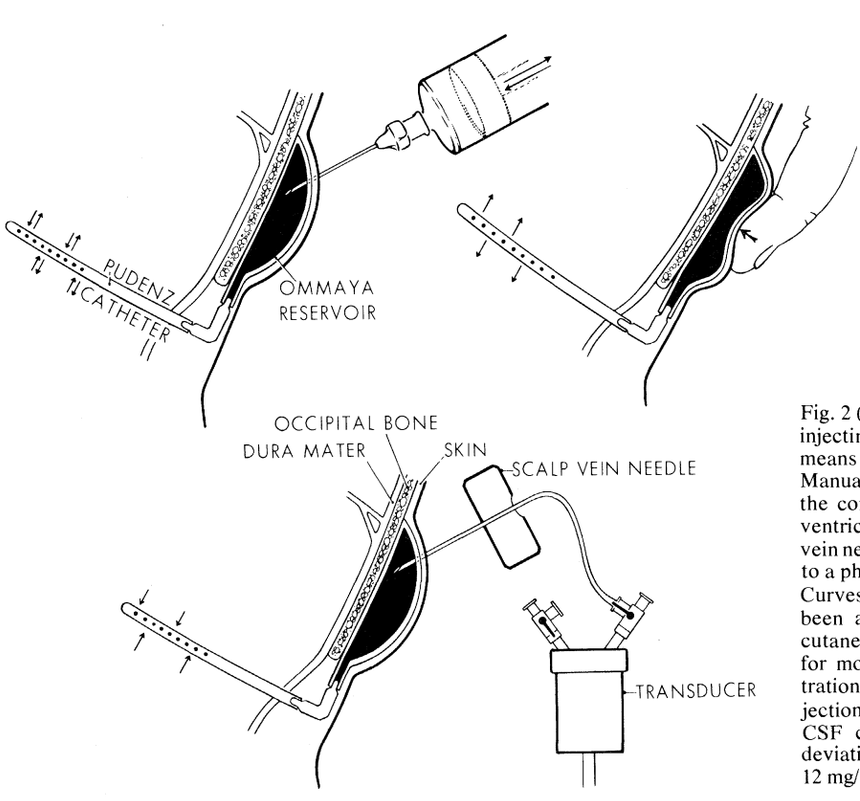


Fig. 1. (Left) Catheter placed in the fourth ventricle and connected to subcutaneous CSF reservoir. (Right) The T-shaped catheter in the spinal subarachnoid space connected to the subcutaneous CSF reservoir.



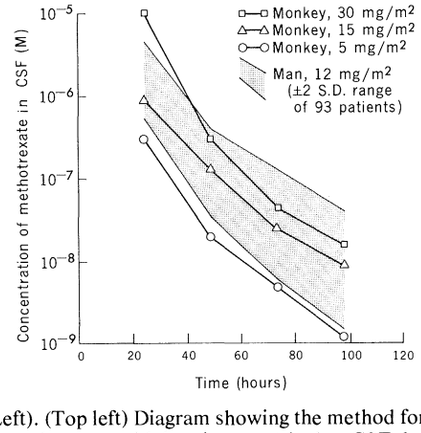


Fig. 2 (Left). (Top left) Diagram showing the method for injecting a drug or withdrawing ventricular CSF by means of a syringe with a 25-gauge needle. (Top right) Manual depression of the reservoir (pumping) to expel the contents into the fourth ventricle. (Bottom) Intraventricular pressure being recorded with a 23-gauge scalp vein needle attached to a soft catheter which is connected to a physiologic pressure transducer. Fig. 3 (above). Curves for the disappearance of methotrexate that has been administered intraventricularly by way of subcutaneous reservoirs in monkeys and man. The curves for monkeys are almost parallel and absolute concentrations are proportional to dosage. Intrareservoir injections of 15 mg/m² methotrexate in monkeys yielded CSF concentrations within the range [± 2 standard deviations (S.D.)] noted in 93 patients injected with 12 mg/m² methotrexate by the same method.

ventricular pressure, and (vi) provides pharmacokinetic data similar to that obtained in man. This primate model may be useful for long-term physiologic, neurochemical, chemotherapeutic, and neurotoxicological evaluations.

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References and Notes

1. R. A. Ratcheson and A. K. Ommaya, *N. Engl. J. Med.* **279**, 1025 (1968).
2. W. R. Shapiro, D. F. Young, B. M. Mehta, *ibid.* **293**, 161 (1975).
3. W. Feldberg and S. L. Sherwood, *J. Physiol. (London)* **120**, 3P (1953); *ibid.* **123**, 148 (1954).
4. P. C. Merker, M. D. Walker, E. P. Richardson, R. F. Shepard, *Excerpta Med. Int. Congr. Ser. No. 311, Proc. Eur. Soc. Study Drug Toxic.* **15**, 3 (1973).
5. T. J. Haley and R. W. Dickinson, *J. Am. Pharm. Assoc.* **45**, 432 (1956); A. C. Palmer, *J. Physiol. (London)* **149**, 209 (1959).
6. M. J. Bresnan *et al.*, *Trans. Am. Neurol. Assoc.* **97**, 204 (1972).
7. A. K. Ommaya, *Lancet* **1963-II**, 983 (1963).
8. J. H. Wood, D. G. Poplack, W. J. Flor, E. N. Gunby, A. K. Ommaya, in preparation.
9. G. DiChiro, S. M. Larson, T. Harrington, G. S. Johnston, M. V. Green, C. J. Swann, *Acta Radiol.* **14**, 379 (1973).
10. S. M. Larson, G. S. Johnston, A. K. Ommaya, A. E. Jones, G. DiChiro, *J. Am. Med. Assoc.* **224**, 853 (1973).
11. A. S. Fleischer, J. M. Patton, G. T. Tindall, *Surg. Neurol.* **3**, 309 (1975).
12. C. DiRocco, D. G. McLone, T. Shimoje, A. S. Raimondi, *J. Neurosurg.* **42**, 683 (1975).
13. J. R. Bertino and G. A. Fischer, *Methods Med. Res.* **10**, 297 (1964).
14. H. J. Hoffman, E. B. Hendrick, R. P. Humphreys, *J. Neurosurg.* **44**, 256 (1976).
15. We thank M. Kostolich, A. Stearns, C. Hawkins, and C. Seay for their technical assistance.
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Memory for Lists of Sounds by the Bottle-Nosed Dolphin: Convergence of Memory Processes with Humans?

Abstract. *After listening to a list of as many as six discriminably different 2-second sounds, a bottle-nosed dolphin classified a subsequent probe sound as either "old" (from the list) or "new." The probability of recognizing an old probe was close to 1.0 if it matched the most recent sound in the list and decreased sigmoidally for successively earlier list sounds. Memory span was estimated to be at least four sounds. Overall probabilities of correctly classifying old and new probes corresponded closely, as if recognition decisions were made according to an optimum maximum likelihood criterion. The data bore many similarities to data obtained from humans tested on probe recognition tasks.*

Almost all experiments on short-term (immediate) memory in animals have been limited to the study of single-item retention, such as the ability to recognize among alternative visual stimuli the one item previously seen (*1*). Results from these experiments have provided important information on time-dependent processes in animal memory and on the effects of irrelevant activities or stimulation during the retention interval on recognition performance. However, they have contributed little information on more complex memory characteristics, such as storage modes, memory scanning rates, span of memory, and retrieval and decision strategies, characteristics that may be better assessed by tasks requiring the retention of multiple, serially occurring items. Such tasks have so far been limited to a few investigations of the ability of monkeys to reproduce in their response sequence the serial order of occurrence of two or three prior stimuli (*2*).

We now present results of tests of item recognition memory of a bottle-nosed dolphin, *Tursiops truncatus*, using serial lists of sounds as long as six items. In concept and method, these tests closely followed serial probe recognition tests given humans (*3, 4*). In the probe recognition test, a list of unique items is presented and followed by a single probe item, which is either an "old" item from the list or a "new" item. The task is to classify the probe correctly as old or new, a memory dependent process. The dolphin studied easily learned the requirements of the multiple-sound probe recognition task, and classified probes as old or new with great accuracy. These capabilities gave further evidence of the impressive auditory learning skills of *T. truncatus* (*5*). Overall, our results for the dolphin were similar to results obtained from human subjects in probe recognition tasks and revealed many of the same capabilities and constraints observed in human performance.

The dolphin tested, an adult female of 11 to 13 years named Keakiko, was the subject in earlier studies of single-item auditory retention (*6, 7*). We tested her twice daily in her seawater tank (diameter, 15.2 m) at the University of Hawaii. Each testing consisted of 30 to 48 discrete probe recognition trials (intertrial interval, 30 seconds). At the beginning of a trial the dolphin heard a highly familiar sound cue. In response, she swam through a channel of four vertically suspended ropes and pressed a "start" paddle 1 m beyond the channel exit, turning the sound cue off. Four seconds later, while passively stationed underwater facing the start paddle, she heard a list of k discriminably different sounds (k = 1, 2, ..., 6) projected from an underwater speaker (Chesapeake J9) located 1.2 m beyond the start paddle. Each sound was 2 seconds long, and successive sounds were separated by 0.5-second silent intervals. After a 1- or 4-second pause, the probe sound, 2 seconds long, was projected from one of two peripheral J9 speakers positioned 1.6 m to the left and right of the center speaker, diagonally facing the start paddle. Adjacent to each peripheral speaker was a response paddle. To respond "old sound (Yes)" the dolphin swam to the peripheral speaker that projected the probe and pressed the adjacent paddle. To respond "new sound (No)" she swam to the silent speaker, the one that projected no sound, and pressed the adjacent paddle. All correct responses immediately yielded a short (0.5-second) familiar conditioned reinforcer sound and then a thrown-fish reward. These were omitted after incorrect responses. Old and new probe sounds occurred with equal probability, each type occurring equally often at each peripheral speaker.

Testing began with single-sound recognition trials (k = 1), a procedure in which the animal was previously trained (*7*), and then proceeded serially, with all testing of k-sound lists completed before testing of $k + 1$ sound lists was begun. Transfer from a list of k sounds to a list of $k + 1$ sounds was accomplished by gradually increasing the duration of the added sound over 30 to 50 training trials until its final value of 2 seconds was reached; transfer was always completed without any disruption in performance.

The sounds in a list, as well as the probe, were selected from a pool of 600 discriminably different sounds that composed six different classes of sounds of 100 sounds each (*8*). The sounds were generated by oscillators (Wavetek) controlled by a minicomputer. A list of k