Chronic Catheterization of Carotid and Vertebral Arteries in Cats.* (24156)

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In investigating drug action on the central nervous system, it is sometimes advantageous to introduce the drug directly into the cerebral circulation, by-passing the systemic vessels. Since certain drug effects can be examined only in the unanesthetized state, a technic permitting injection into intact, freely moving animals is essential. In this way, valuable observations can be made upon the neurologic status of the animal, its general behavior, or even its performance in a psychological test situation, and electrical activity of the brain recorded. Furthermore, because drug effects vary with dose, it is helpful to have a permanent preparation in which different doses of drug may be tested repeatedly over weeks with a single animal serving as its own control. In the following a technic is described for permanently implanting carotid and vertebral catheters in cats, with a consideration of how such a preparation can be of value in studying central nervous system drug effects.

Method. Carotid catheterization: in cats under pentobarbital anaesthesia, one common carotid artery is exposed aseptically and the largest muscular branch at the level of the thyroid cartilage divided as far distally as possible. The carotid is occluded above and below this branch, which is then slit transversely, and a catheter‡ introduced into the muscular branch and advanced up the common carotid for approximately 1-2 cm. It is then tied firmly in place at the bulb with several ligatures around the muscular branch and the other end brought out, by means of a hubless trocar, through a stab wound between the scapulae. *Vertebral Artery*: a longitudinal incision is made to the left of the midline over the lower neck, and the left subclavian artery and brachial plexus exposed and identified, as well as the thyrocervical trunk, costocervical trunk and the vertebral artery. The axillary artery, costocervical trunk, and thyrocervical trunk are then ligated, the latter as far distally as possible. With the subclavian artery temporarily occluded proximally, the thyrocervical trunk is opened transversely and the catheter inserted into it towards the heart until it enters the subclavian and its end rests about at the point of origin of the vertebral artery, where it is tied in place. Injections are thus made into the subclavian at point of origin of the vertebral, with the other branches of the subclavian in the vicinity (axillary, thyrocervical trunk, and costocervical trunk) tied off. It will be apparent that the left side is suitable for such a procedure anatomically, but the right side is much less so because of the immediate proximity to the vertebral of the right common carotid artery. The external end of the catheter is then attached to a modified polyethylene tubing adapter (Clay-Adams). Its hub is shortened and screw threads made in its base externally. The cavity of the hub is then filled with a soft rubber plug suitable for needle puncture and a specially made collar with a central hole is screwed down over the hub to keep the rubber firmly in place. The apparatus is then mounted in a plastic yoke containing holes through which it may be sewed to the underside of a closely fitting plastic animal jacket. Injections are made by inserting a #27 short hypodermic needle through the diaphragm. It is possible to give the injection (usually 0.1 cc-0.2 cc) quickly without disturbance, and to withdraw the needle while observing and testing the animal; or one may attach the catheter to a long polyethylene hose of the same caliber, using a short section of #27 needle as a link, and

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‡ Size PE 10 Clay-Adams polyethylene tubing prepared with a small bulb 2 cm from one end and coated with Siliclad (Clay-Adams) to minimize clotting. Catheters were sterilized in benzalconium chloride and washed thoroughly with sterile saline.

make intracarotid injections from a distance in the undisturbed cat. The catheter is flushed with sterile saline before and after injections of the drug and is filled with heparin at the end of each test period. Electrodes have been implanted over the cerebral cortex of the same animal, making it possible to record the EEG during the drug action.

Results. A number of drugs have been employed in this preparation, and the neurological, behavioral, and EEG effects noted. The effects of intracarotid sodium pentobarbital will serve as an instructive example.

Intracarotid sodium pentobarbital was given over a wide range of doses to establish threshold, minimal effects, etc. Because of its relatively slow rate of elimination, only one injection was given each day. In cats weighing 3-4 kg, doses of 0.2 mg or less gave no effect. Minimal effective doses (0.25-2.0 mg) produced a characteristic syndrome of circling to the side of the injection, accompanied by neglect of the contralateral homonymous visual field. Certain of the cats invariably showed simultaneous relaxation of the ipsilateral nictitating membrane. Duration of symptoms depended on dose. Doses in the intermediate range (2.0 to 20.0 mg) produced the above effects plus weakness of contralateral extremities, manifested by feeble withdrawal from noxious stimuli and inadequate hopping and placing reactions. Larger doses (20-30 mg) produced evidence of marked motor impairment of the contralateral side—a rather complete hemiplegia with increased extensor tone in the contralateral fore and hind limbs, and increased deep tendon reflexes. The cat fell to the contralateral side due to lack of support. This was accompanied by pronounced curvature of the body to the ipsilateral side and adversion of the head to that side as well.

With doses in the intermediate range, generalized effects became apparent in a minute or two, once the drug had sufficient time to recirculate and affect the rest of the brain. This usually manifested itself by pronounced playfulness and staggering gait, sometimes accompanied by drowsiness but never by excitement. With larger doses the cat became

FIG. 1. The 2 channels record EEG from homologous areas of each hemisphere and show marked EEG asymmetry resulting from administration (in 1 mg doses) of 9 mg of pentobarbital into left common carotid artery. Record from left hemisphere shows a series of spindle bursts, as seen in moderately deep barbiturate anaesthesia. Record from right hemisphere is that of alert wakefulness and has not changed significantly from control period taken before drug administration.

stuporous or unconscious. After the largest doses employed (75 mg) unconsciousness supervened so rapidly that there was scarcely time to make any useful observations.

It was possible to record the EEG while the injections were being made remotely, with the cat undisturbed but observed. Sodium pentobarbital produced marked EEG asymmetry, with the side ipsilateral to the injections first showing barbiturate induction activity (high voltage fast waves, 18-20/sec.), then barbiturate sleep (slow waves and spindles). During this time, the record from the contralateral hemisphere was that of the normal waking cat (Fig. 1). When injections were stopped, the process reversed itself, passing back from "sleep" through "induction" to normal. Recovery was much more rapid than that observed after intravenous injection. EEG effects correlated well with behavioral observations, since the animal showed contralateral hemiparesis when EEG effects were maximal; both recovered together.

Complications of the method. If blood passes back up the catheter and remains for any length of time, it may clot and permanently occlude the catheter. Hence it is important to prevent leaks in the system and to insure that after each experimental session the catheter is filled with heparin solution. Although the cats are not particularly inclined to disturb the catheter where it is exposed externally, they may scratch the region or attempt to groom it, so that it is essential to avoid any loops in which the animal may catch its paw. Consequently, the external end of the catheter may be plugged with wire

and left free, or attached to the special adapter and protected beneath a plastic coat. We have had no infections and there has been no suggestion of embolization of the brain at time of injection or evidence of such upon inspection of the brain at autopsy.

In those animals in which intra-arterial clotting ultimately took place, the thrombus has been located within the carotid a little beyond the tip of the catheter, rather than at the point of entrance of the catheter into the carotid where it might have been anticipated. In one instance, the unilateral drug effects disappeared and were replaced by evidence of discomfort on injection. The animal was sacrificed and india ink injected into the catheter immediately before death. A thrombus occluded the common carotid just beyond the catheter tip; the ink had traveled centripetally to the junction of the 2 carotids, up the opposite carotid and into both hemispheres of the brain by crossing over in the circle of Willis. In a second instance, the threshold for the unilateral effects of several different drugs suddenly increased 4-5 fold, while there was no change in the cat's susceptibility to their general (recirculation) effects. At autopsy, it was found that the internal carotid was thrombosed at its origin, and the channel to the external carotid (and thus through the internal maxillary artery and carotid plexus to the circle of Willis through the orbital fissure) was partly occluded as well. Apparently full patency of both internal and external carotid arteries is important in obtaining low-threshold unilateral effects, but that if the occlusion is only partial, unilateral effects can still be obtained but with corresponding decrease in unilateral versus generalized drug effects.

Certain side effects of the drug injection, such as relaxation of the ipsilateral nictitating membrane and licking and gagging, appear to be due to irrigation of the superior cervical ganglion and the tongue and throat respectively. By placing the tip of the catheter beyond the source of blood supply to these structures, one can observe central neurological effects alone.

It was feared that in the case of the indirect vertebral catheterization, ligation of the left subclavian artery and costocervical and thyrocervical trunks might produce ischaemia of the left forelimb. This has not been the case, but a reversible paraparesis involving both lower extremities has been noted, apparently from diminution of the blood supply to the spinal cord at the cervicothoracic junction.

Discussion. Use of the carotid route for administration of neurotropic drugs to unanaesthetized man was begun by Wada(1). Injections were made by percutaneous carotid puncture and resulting neurological and psychological alterations observed. This method has promise but is naturally limited to safe drugs selected primarily for possible therapeutic effect. Recently, this technic has shown itself especially useful in the lateralization of speech function in man(2).

The intra-arterial route has been employed in experimental animals for a variety of purposes. The carotid loop technic permits intracarotid injections in trained, unanaesthetized dogs(3,4,5). A single injection of the long-lasting anticholinesterase, DFP, into the rabbit carotid produced significant effects upon the EEG(6) and behavior(7). These injections were made under local or short-acting barbiturate anaesthesia, and the drug effects lasted long enough to allow examination of the freely moving or curarized animal. For observation in the acute preparation of short-lasting drug effects or for observing the effects of several repeated injections, a carotid T-tube or catheter has been employed(8,9,10). Subsequent use has been made of single or repeated intracarotid or intravertebral injections in animals immobilized by brain stem section or curarization(11,12,13,14), or anaesthesia(14,15,16).

Short-term chronic carotid catheterization in the rat was employed in the study of ADH release(17), and a technic for long-term chronic carotid catheterization has been successfully devised by Rudolph and Paul(18) for the repeated measurement of intra-arterial pressure in dogs. An indirect technic for intravertebral injection in guinea pigs was employed by Diamant(19), and involved retrograde (centripetal) injection into either caro-

tid. with both carotids occluded further distally. This was done under local anaesthesia and sometimes a single animal was used more than once, but the method carried the disadvantage that it required injection of a large fluid volume with simultaneous clamping of both common carotids, measures which alter cerebral hemodynamics profoundly.

With our method, we have found it possible to prepare cats with chronic catheters and to maintain them for long periods. Such preparations afford certain distinct advantages in the study of drug action upon the nervous system. The preparation is very sensitive. Whereas a drug-induced decrease of total motor output of both cerebral hemispheres of perhaps 10% would scarcely be detectable merely by observing the animal, such a decrease in only one hemisphere becomes readily apparent, since by imbalance of the output, the animal turns to one side when it attempts to walk.

The presence of a blood brain barrier to different agents may be studied. The total dose of drugs given systemically to the intact animal may be limited by undesirable side effects (such as respiratory paralysis in the case of curare) or by very rapid destruction or inactivation. The present method makes it possible to administer large doses to the brain with comparatively little systemic effect, and the relative capacity to penetrate the blood brain barrier is increased. Furthermore, since the drug may also irrigate the superior cervical ganglion, which lacks this barrier, and thus affect the nictitating membrane, it is possible to compare the effects of the drug upon central and peripheral synapses simultaneously.

The latency within which drugs act upon the brain may be measured precisely, since the drug blood level in the brain is very high initially during a single circulation time, and falls sharply immediately thereafter. Furthermore, the recovery rate of nervous tissue may be ascertained, since after the injection the concentration gradient is away from the brain only.

The locus of drug action may be more precisely determined, since it is possible to administer drugs predominantly to the hemispheres, with little reaching the lower brain stem, or vice versa. Furthermore, since the hemispheral action is predominantly unilateral, the other hemisphere serves as a control against systemic or non-specific effects. Some drugs may act upon the nervous system only indirectly, by releasing other chemical substances or after being changed chemically themselves. Since these all involve recirculation, this phenomenon will be revealed by the absence of lateralized effects.

Since a single animal can be examined repeatedly over a period of months, it is possible to give different doses of a drug and establish dose-response curves, or to administer different drugs and make quantitative comparisons. Lastly, since the animals are healthy and freely moving it is possible to test them in a variety of ways, neurologically and psychologically, to photograph their movements and behavior, and to record from the surface or depths of the brain by means of implanted electrodes.

Summary. 1. A technic is described which makes it possible to administer drugs into the carotid or vertebral circulations of intact, freely moving cats repeatedly over a period of weeks or months. Observations and photographs have been made of the resulting neurological or psychological effects, accompanied in some instances by simultaneous electrical recording from the cortex and deeper structures. 2. The effect of intracarotid sodium pentobarbital is described as an example. This has produced ipsiversive circling, relaxation of the ipsilateral nictitating membrane, and contralateral paralysis, spasticity, and homonymous hemianopsia, the effects varying with dose. Unilateral EEG changes, characteristic of barbiturates, accompany the neurological symptoms and signs. The latency of onset is very short and all the effects are fully reversible in 5-10 minutes. The advantages of the technic are discussed.

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Immunochemical Studies with Radioactive Isotope, Similarity and Difference Between Serum Vitellin and Lipovitellin. (24157)

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The serum of laying hens was first distinguished from that of non-laying hens and of cocks by Sasaki(1) who used immunological precipitin tests. Roepke and Bushnell(2) using immunological methods detected traces of protein similar to ovovitellin in serum of cocks, and more in laying hens. Lakowski (3) found in plasma of laying hens a phosphoprotein which he named serum vitellin since its chemical nature is similar to ovovitellin. Recently Hosoda and his associates(4), using immunological technic determined the vitellin content in blood serum of hens and found that its titer corresponds to ovarian activity of hens. Wormall and his co-workers (5,6) studied the chemical composition of lipovitellin-antilipovitellin precipitates using radioactive isotopes. The present work was undertaken to elucidate the relationship between serum vitellin and egg yolk lipovitellin, using immunochemical methods with radioisotope.

Materials and methods. Antigens. 350 μc P^{32} as Na_2HPO_4 was injected subcutaneously into laying hens. After 24 hours the hens were sacrificed, and P^{32}-containing serum and yolk solution taken up as antigens in 10% NaCl. *Antiserum* to serum of laying hens was prepared by immunizing rabbits with non-radioactive serum of laying hen, and absorbing the species-specific antibodies with cock serum. This type of absorbed-antiserum has been used by Hosoda *et al.*(4) for determination of serum vitellin. Anti-yolk serum was prepared by immunizing rabbits with a suspension of non-radioactive yolk. This antiserum had a considerable amount of species-specific antibodies, absorbed with cock serum. Lipovitellin used as antigen was prepared as described by Chargaff(7). Antilipovitellin serum was prepared by immunizing rabbits with a suspension of nonradioactive lipovitellin. Anti-lipovitellin serum also had considerable amount of species-specific antibodies, which were absorbed with cock serum. Phenol was added in a concentration of 0.25% to all antisera for preservation, and these antisera were stored at 3-5°C. *Precipitin tests.*