and was not.

Summary and conclusions. 1. Chlorpromazine and dibenzyline were tested in mice for their ability to afford protection against a variety of bacterial toxins. 2. Premedication and treatment with chlorpromazine significantly decreased the mortality from endotoxins derived from *Escherichia coli* and *Salmonella typhosa*. Similar treatment with dibenzyline decreased the mortality from toxins derived from *Shigella dysenteriae, Aerobacter aerogenes, Pasteurella pestis* and *Clostridium tetani*. 3. Chlorpromazine afforded significant protection when administered 2 hours following the injection of endotoxin derived from *Escherichia coli*. Premedication was not necessary. 4. Premedication with chlorpromazine increased the mortality from the toxin of *Clostridium perfringens*, likewise premedication with dibenzyline increased the mortality from the endotoxin derived from *Salmonella typhosa*.

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1. Fine, J., *The bacterial factor in traumatic shock*, 1954, C. C. Thomas, Springfield, Ill.

2. Aub, J. C., Brues, A. M., Dubos, R., Kety, S. S., Nathanson, I. T., Pope, A., and Zamecnik, P. C., *War Med.*, 1944, v5, 71.

3. Nelson, R. M., and Noyes, H. E., *Surg. Forum Clin. Cong. Am. Coll. Surg.*, 1952, W. B. Saunders, p474.

4. Wiggers, H. C., Goldberg, H., Roembild, F., and Ingraham, R. C., *Circ.*, 1950, v20, 179.

5. Hershey, S. G., Guccione, I., and Zwiefach, B. W., *Surg., Gynec. and Obst.*, 1955, v101, 431.

6. Millican, R. C., and Stohlman, E. F., *Am. J. Physiol.*, 1956, v185, 195.

7. Reilly, J., Campagnon, A., Tournier, Paul Du, Buit, H., *Ann. Med. Par.*, 1954, v55, 5.

8. Abernathy, R. S., and Halberg, F., *Proc. Cent. Soc. Clin. Res.*, 1955, v28, 7.

9. Brancadoro, C., and Visconti, S., *Bior. ital. Chir.*, 1954, v10, 830. Abst. *J.A.M.A.*, 1955, v157, 1161.

10. Adriani, J., and Kerr, M., *South. Med. J.*, 1955, v48, 858.

11. Wright, G., personal communication.

12. Noyes, H. E., Pulaski, E. J., and Balch, H. H., *Ann. Surg.*, in press.

13. Ajl, S. J., Reedal, J., Durrum, E. L., and Warren, J., *J. Bact.*, 1955, v70, 158.

14. Webster, M. E., Sagin, J. F., Landy, M., and Johnson, A. C., *J. Immunol.*, 1955, v74, 455.

15. Branham, S. E., and Habel, K., *ibid.*, 1946, v54, 3.

16. Editorial, *Lancet*, 1955, v1, 337.

17. Kelan, M., and Zweifach, B. W., *Surg., Gynec. and Obst.*, 1954, v99, 707.

18. Thomas, L., *Fed. Proc.*, 1956, v15, 618.

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Concentration of ACTH in Cavernous Sinus and Peripheral Arterial Blood in the Dog.* (22563)

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Collection of effluent blood from the pituitary has been impractical because of its position in the skull and the early divergence of cerebral venous drainage into many channels. Since blood drains directly from the pituitary into the cavernous sinus, at least in some species, samples of blood from this location, although contaminated by blood from the ophthalmic veins, would be expected to contain a high concentration of pituitary hormones (1). The present paper describes a simple and reliable technic for obtaining blood from the

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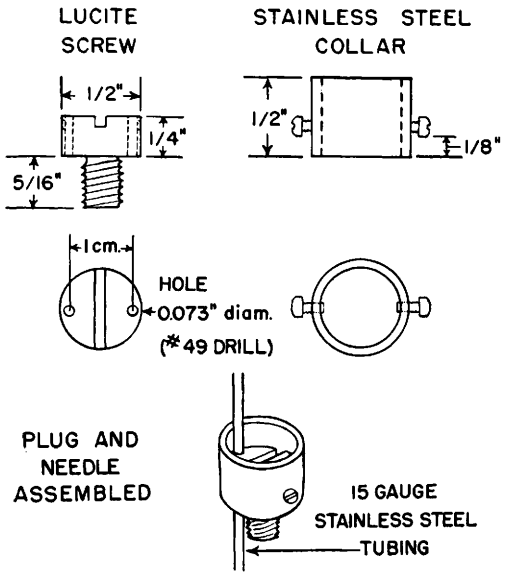

FIG. 1. Plug and guide needle for cavernous sinus puncture.

cavernous sinus in the dog, and presents evidence that the concentration of ACTH in this blood is much higher than that found in peripheral arterial blood.

Materials and methods. Adult mongrel dogs weighing 10-14 kg were used in this study. They were maintained on a diet of Ken-L-Biskit.§ Penicillin, 300,000 units daily, was given intramuscularly for one week after the animals were operated upon. The plastic button with a screw stem and stainless steel collar used to hold the guide needle in place is illustrated in Fig. 1. The stereotaxic machine used in these studies is described in detail elsewhere(2). Implantation of the plastic button was performed under aseptic conditions using intravenous pentobarbital anesthesia. The dog's head was placed in the stereotaxic instrument, and a curved incision was made through the scalp. The incision started in the mid line between the eyes and as it extended posteriorly it curved to one side, passing 3 cm lateral to the mid line opposite the center of the head, and returning to the mid line posteriorly, ending about 3 cm anterior to the occipital protuberance.

A lateral skull x-ray was taken and a reference point was located at the junction of the sloping inner table of the sphenoid bone and the floor of the optic foramen. The carrier of the instrument was centered above a point 1.3 cm posterior to the reference point. The previously outlined skin flap was elevated and retracted. Using the instrument, a hole 7/32 of an inch in diameter was drilled in the mid line down to the dura. The hole was tapped with a square ended ¼ inch #20 thread tap, and the button was inserted and tightened, so that a line drawn through the two holes in the button was perpendicular to the sagittal suture. Two short pieces of stainless steel wire were passed under the head of the screw, one forming a loop toward the front and the other toward the back of the head. The skin flap was then replaced after a round hole was cut in it to accommodate the head of the screw, and the ends of the stainless steel wire were brought thru the skin close to the hole and tied to each other, thus securing the skin in place about the plastic button. The carrier was next moved 5 mm to one side, a #49 drill was inserted, and using the holes in the plastic button as a guide, a hole was drilled thru the skull. A 3 inch length of 15 gauge stainless steel tubing‖ with a square-cut smoothed end was inserted thru the hole and thru the brain to, but not thru, the superior wall of the cavernous sinus. This structure was identified by feeling an elastic resistance to the tip of the needle. The stainless steel tubing was cut off *in situ,* leaving ⅛ of an inch of tubing projecting above the button. The stainless steel collar was next slipped over the plastic button and the set screws tightened to hold the needle in place. A bent piece of 18 gauge hypodermic needle was inserted in the top of the 15 gauge needle as a trochar, the wound was sutured, and the animal was allowed to recover for at least one week. Guide needles were put down to only one of the 2 cavernous sinuses at a time. If, at a later date, it was desired to tap the other cavernous sinus, a hole was drilled on the outer side and a needle was inserted in the same manner as on the first side. When the

§ Quaker Oats Co., Chicago, Ill.

‖ Obtained from MacGregor Instrument Co., Needham, Mass.

animals had fully recovered, cavernous sinus blood was obtained by removing the trochar, and inserting a sterile 18 gauge lumbar puncture needle down the guide needle and into the cavernous sinus. Blood was collected into heparinized syringes, care being taken to exert very little pressure on the syringe so as not to reverse the flow of blood in the sinus. In a few instances, the dog was heparinized and laid on his back. Cavernous blood was then drained by gravity thru the lumbar puncture needle into a test tube. The buttons were well tolerated, and the small amount of reaction around the device subsided in about a week.

In the present studies, 25 cc of cavernous sinus blood and 50 cc of femoral arterial blood were drawn simultaneously from normal and adrenalectomized dogs under ether anesthesia. The animals were subjected to various degrees and durations of surgical stress. Samples were also obtained from the cavernous sinus of unanesthetized unrestrained dogs. Puncture of the sinus is occasionally somewhat painful, but the pain, when present, lasts only for an instant, and thereafter the animals appeared to show no ill effects from the puncture. Subsequent removal of the puncture needle was not associated with significant hemorrhage in any instance, presumably because the elastic wall of the sinus was not prone to leak and because the pressure within the sinus was so low. As many as 5 samples were taken at 2-3 day intervals from some of the dogs, and aside from the anemia due to blood removed, no untoward effects were noted.

Two dogs in whom cavernous sinus guide needles had been inserted were subjected to cannulation of the right adrenal according to the technic of Hume and Nelson(3), and the animals were permitted to recover. Three days later, adrenal venous blood samples were obtained while the puncture needle was inserted into the cavernous sinus. Adrenal venous samples were taken before, and 5, 10, and 30 minutes after, the insertion of the needle into the cavernous sinus.

One dog in whom a cavernous sinus guide needle had been implanted was subjected to laparotomy under pentothal-induced ether anesthesia. A cavernous sinus blood sample was obtained, and immediately thereafter his pituitary was removed thru the trans-buccal approach. One half hour later, another cavernous sinus blood sample was obtained.

TABLE I. ACTH Content (Milliunits per 100 cc Plasma) of Cavernous Sinus and Femoral Artery Blood Samples Drawn Simultaneously at Arbitrary Times during Various Experimental Conditions.

Dog	ACTH content, milliunits/100 cc plasma	
	Cavernous sinus blood*	Femoral artery blood*
311	18	4.0
X	50	2.0
18	12	0 †
115	40	10
312	18	2.0
148	20	0 †

* ACTH content determined by inj. the plasma from 25 cc of cavernous sinus blood and 50 cc of femoral artery blood.

† ACTH could not be detected in the volume of plasma tested.

All cavernous and arterial samples were placed in heparinized tubes, centrifuged immediately, and the plasma stored in the frozen state until used. Determination of the ACTH content of these samples was performed by the technic of Nelson and Hume(4). The 17-hydroxycorticosteroid content of the adrenal venous blood was determined by the method of Nelson and Samuels(5).

Results. The ACTH content of the simultaneously obtained cavernous sinus and arterial blood samples is listed in Table I. It is apparent that the concentration of ACTH in the cavernous sinus blood was much greater than it was in the femoral artery blood. The ACTH concentration in the cavernous sinus blood of the animal subjected to laparotomy and hypophysectomy was 28 milliunits per 100 cc of plasma before hypophysectomy, and was not detectable one-half hour after. It would appear, therefore, that the substance being assayed was pituitary in origin. Repeated cavernous sinus samples during other surgical procedures without removal of the pituitary show consistent high levels for at least 60 minutes. The effect of cavernous sinus puncture on adrenocorticosteroid output is illustrated in Table II. Insertion of the

TABLE II. Adrenal Venous Blood 17-Hydroxycorticoid Output (μg/Min.) in Response to Puncture of the Cavernous Sinus.

	Dog No. 312	286
Control	3.3	.2
5 min. after puncture	15.6	12.4
10 " " "	1.2	9.9
30 " " "	2.3	.2

needle in these 2 dogs was associated with a definite but small and transient rise in corticoid output.

Discussion. Hume and Nelson(3) have recently published a technic for obtaining adrenal venous effluent blood in the unanesthetized animal. This method for studying adrenal hormone output has two advantages over the present technic for studying pituitary hormone secretion. In the first place, the adrenal has a single effluent vein, making it possible to obtain all of the undiluted adrenal venous blood. In the dog the cavernous sinus at the level at which it is tapped drains not only the pituitary but also the ophthalmic veins. Secondly, the present method of obtaining cavernous sinus blood does not permit measuring the rate of pituitary blood flow. Therefore, actual measurements of ACTH output per unit time are as yet beyond the reach of this method. In some species, much of the pituitary venous blood passes through the sphenoid bone via the emissary veins. Detailed descriptions of the venous outflow from the pituitary in the dog are not available. However, the present data showing a much greater concentration of ACTH in cavernous sinus than in peripheral blood indicate that in the dog at least a part of the pituitary drainage is into the cavernous sinus. Furthermore, the emissary vein in this species is extremely small and often altogether absent. The advantage of the present technic lies in the high concentration of ACTH, and presumably of other pituitary hormones, in the cavernous sinus blood. This high concentration permits the use of smaller volumes of blood for hormone measurement, and may make possible the detection of ACTH under circumstances where its detection in the peripheral blood is difficult or impossible. Furthermore, although insertion of the cavernous sinus needle has been demonstrated to be a mild stress, it is a short lasting one. If the needle is inserted and left in place for 20-30 minutes, the effects of its insertion will have worn off so that cavernous sinus blood can be obtained in the unanesthetized, unrestrained normal animal. Studies of the effect of trauma and various other conditions on cavernous sinus ACTH content are under way in this laboratory at present.

Summary. A simple and reliable method of obtaining blood from the cavernous sinus of the dog has been presented. The level of ACTH in this blood under a variety of conditions has been found to be many times higher than the concentration in peripheral arterial blood. Hypophysectomy causes a fall to zero in the level of ACTH in this blood. With this technic, insertion of the needle into the cavernous sinus is shown to be a definite but transient stress.

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1. Wislocki, G. B., and King, L. S., *Am. J. Anat.*, 1936, v58, 421.

2. Hume, D. M., and Ganong, W. F., *Electroencephalography and Clin. Neurophysiol.*, 1956, v8, 136.

3. Hume, D. M., and Nelson, D. H., *Surgical Forum, Am. Coll. Surg.*, 1954, v5, 568, W. B. Saunders Co., 1955.

4. Nelson, D. H., and Hume, D. M., *Endocrinology*, 1955, v57, 184.

5. Nelson, D. H., and Samuels, L. T., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 519.

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