ture media is of interest. It suggests that such antigens are released by the cell damage and breakdown seen in routinely cultured spleen suspensions(12) and that, with anoxic necrosis avoided in hyperoxic cultures, antigen is not released.

Summary. In hyperbarically oxygenated cultures, using phytohemagglutinin as a mitogen, spleen and lymph node slices showed great proliferative activity for a 12-day period. Nine-tenths of the yield was obtained in the first two days. Seventy-five to 80% of the cells produced were lymphocytes. The antigenicity of these culture-grown cells did not appear to be diminished as tested by their ability to produce second-set skin graft rejection. Culture media contained no demonstrable antigen.

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Methods for Obtaining the Venous Outflow from the Hypothalamus and Hypophysis.* (31096)

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Control of the anterior pituitary by neurohormones from the hypothalamus is well established. The mechanisms by which the control centers are regulated, however, are quite obscure. While there is general agreement that target-organ hormone feedback (*i.e.*, steroids on gonadotropin production and/or release) is important, some authors(1) visualize an "internal feedback" loop whereby the hormones of the anterior pituitary modify hypothalamic activity. Obviously a vascular link from the pituitary to the hypothalamus is a vital part of such a concept. Anatomical evidence for proximally direct (*i.e.*, toward the hypothalamus) venous drainage of pituitary blood was reported by Popa and Fielding(2). Later studies have concentrated mainly upon the well-known distal drainage of the hypophyseal portal system(3). Recent observations in the living cat and dog(4) have verified proximal blood flow and give indirect support for an internal feedback theory.

According to Peele(5) the major portion of venous blood draining the hypothalamus empties into the great vein of Galen (*V. Cerebri magna*). Demonstration of higher titers of pituitary hormones in the effluent of this vessel, compared with peripheral blood, would give further support for proximal transfer.

The present study deals with: (1) a method for intradural sinus cannulation of the great

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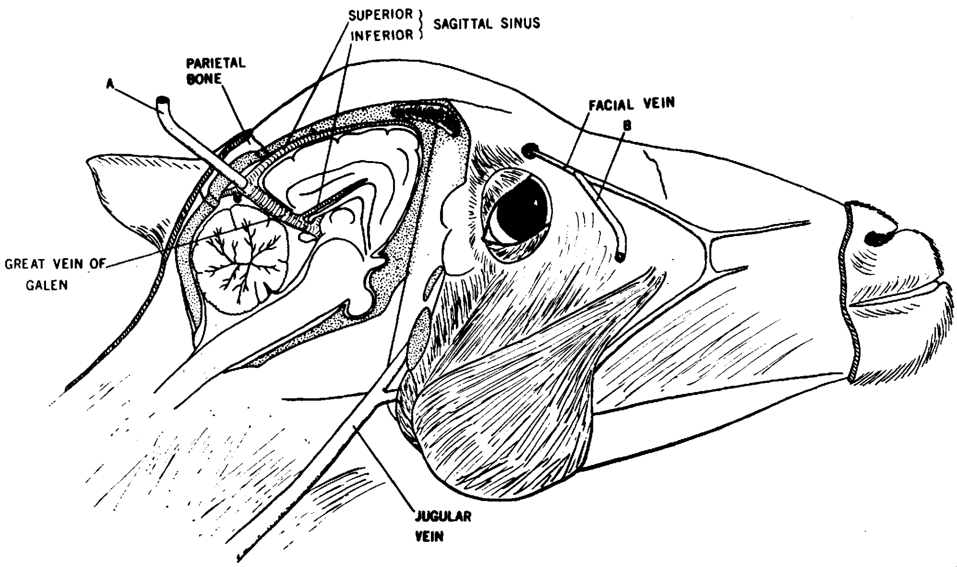

FIG. 1. Diagrammatic presentation of cannulae placement in the great vein of Galen (A) and the facial vein (B) of sheep.

cerebral vein (of Galen); (2) a method for obtaining cavernous sinus blood *via* the deep facial vein, and (3) assay of luteinizing hormone activity in the plasma from these vessels in sheep.

Materials and methods. White-faced Western sheep were used for this study. The animals were initially anesthetized with 6% pentobarbital *via* an ear vein and then placed in a horizontal position on the operating table, with the head slightly above the longitudinal axis of the body. A catheter was placed in the large medial cubital vein in order to supplement periodically the anesthetic. Ptylism was controlled by administration of 60 mg atropine sulfate.

A skin line incision was made about 2 cm medial to the anterior angle of the eye and the deep facial vein was exteriorized. The vessel was then ligated and a cannula (PE 200) was inserted proximally to a point just distal to its entrance into the skull (supraorbital foramen) (Fig. 1). The facial vein is large in sheep and communicates with the cavernous sinus *via* the opthalamic vein. Since there are no valves in these vessels, the direction of blood flow can be distal as well as proximal.

In sheep, the great vein of Galen (formed by union of the right and left deep cerebral veins) passes upward and backward behind the splenium of the corpus collosum and is continued as the straight sinus to join the superior sagittal sinus approximately 1 cm rostral to the confluens sinus (torcula of Herophili). The exposure of the superior sagittal sinus was accomplished by removing a mid-sagittal portion of the parietal bone (approximately 1 cm on either side of the mid-line and about 3 cm long) (Fig. 1). A small incision was made in the dorsal wall of the sinus starting at the junction of the confluens and carried forward approximately 2 cm. The cut edges of the sagittal sinus were reflected and the orifice of the straight sinus located by gentle probing. A siliconized polyethylene tube (PE 200) was used for the cannulation since it approximates the diameter of the vessel lumen and does not impede normal blood flow. The catheter was introduced into the straight sinus and continued forward into the great vein of Galen to the point where this vessel bifurcates into the right and left deep cerebral veins (Fig. 1). Care must be taken with the cannulation procedure since the venous wall is very thin. After proper positioning of the cannula, the sagittal sinus was carefully covered with gelfoam to prevent hemorrhage. Following collection of blood samples, the animals were

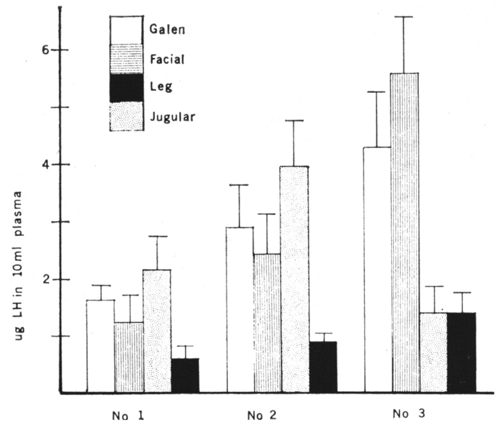

FIG. 2. Amount of LH found in plasma obtained from various veins in 3 sheep. Vertical lines indicate standard error for groups of 4 to 6 assay animals.

sacrificed and the position of the cannulae was confirmed. Repeated approaches to the great vein of Galen have established that the opening of the straight sinus into the superior sagittal sinus is relatively constant in sheep.

Timed blood samples were obtained from the Galen and facial veins as well as by syringe withdrawals from leg and external jugular veins. Following collection, the heparinized blood was spun in a refrigerated centrifuge and the plasma was separated and stored frozen until assayed.

The plasma concentration of luteinizing hormone (LH) was determined by the ovarian ascorbic acid depletion (OAAD) method (6). One and one-half or 2 ml of plasma were administered *via* the tail vein to groups of 4 to 6 animals. With some samples, toxic reactions were noted and required a very slow administration of the plasma. Standard LH (NIH-LH-S5)‡ was used as a reference, and at least 2, but generally 3, doses were used in each assay to construct log-dose response regression curves. From these slopes, the amount of LH in the plasma samples was estimated.

Assay results. Fig. 2 shows the amount of LH in leg (cubital), external jugular, Galen and facial samples, taken from 3 different sheep. Sheep No. 1 was in the third month of gestation, with a single fetus in the uterus and a single large corpus luteum in one ovary. Sheep No. 2 was used 5 weeks after delivery of a lamb that did not survive. Each ovary contained several follicles as well as a regressing corpus luteum. Sheep No. 3 was a young castrate ram. Our attempts to demonstrate LH activity in the blood of an intact ram failed.

The castrate ram had the highest LH value in Galenic and facial vein samples, while the pregnant ewe had the lowest. The number of our samples is not large enough to allow any comment on the significance of these differences. The only important point for immediate concern is that samples from the great vein of Galen have significantly ($p<.05$) higher LH activity than samples of peripheral (leg vein) blood. Differences between facial vein and Galenic samples were not significant. There is here, however, the added problem of flow rate and the question of its influence on the amount of LH activity picked up. The flow rate (ml collected/minutes of collection time) for the great vein of Galen varied between 5.3 and 11.6 ml/min and for the facial between 5.4 and 11.4 ml/min. The flow in the former cannot be determined accurately, however, since the cannula cannot be secured in the vessel. Flow rates for the jugular and leg veins were not obtained.

Discussion. The venous drainage of the ventral portion of the diencephalon by way of the basal veins is well documented in the literature(5,7). Since these vessels have extensive communications with the Galenic system, there seems little doubt that the great vein of Galen contains a major portion of the blood draining the hypothalamic region. The fact that this vessel reaches a point near the surface of the brain makes it an ideal choice for experimentally obtaining venous effluent from the hypothalamus. The intradural sinus approach as outlined in the present report is unique in that it does not require opening of the cerebrospinal (C.S.F.) compartment and thus does not disturb the C.S.F.-blood pressure relationships.

A vascular link between hypophysis and hypothalamus would be of particular importance in considering proximal blood flow from the pituitary stalk region. Anatomical evi-

‡ The standard LH was generously supplied by the Endocrinology Study Section of NIH.

dence for such a connection was demonstrated by Popa and Fielding(2). With the many studies relative to the pituitary control by the hypothalamus, the study of pituitary stalk vasculature was renewed(8), but only distal drainage of blood in the portal system was emphasized. Recently(3,4) interest in a proximal flow has reappeared. Functional significance of an internal feedback concept has been suggested by reports of David, Fraschini and Martini(9) and Corbin and Cohen (10), that exogenous LH implanted into the hypothalamus lowers pituitary and plasma LH in intact and castrate male and female rats. Furthermore, Johnson and Nelson(11) find considerable LH activity in all areas of the hypothalamus. The present finding of LH activity in blood draining the hypothalamus offers an explanation of how the LH gets into this region of the brain and adds to our conviction that an internal feedback theory is worthy of serious consideration.

We must add that the present findings do not prove that pituitary hormones ascend into the brain. Among other complications, there is the possibility that the assay we are using is not specific for LH alone. Indeed, it readily measures endogenous LH in the assay animal, released *via* hypothalamic releasing factor, which could be in the blood we are testing. Pelletier(12) has reported LH activity in plasma from hypophysectomized ewes. Similar reports have appeared for other species (13,14). However, evidence has been presented that in these cases increased releasing factor, secreted following hypophysectomy, was being detected. This same material might possibly be interfering with our assays and more rigorous tests will be necessary before we can unequivocally state the origin of the activity we measured.

LH in cavernous sinus blood from sheep has been demonstrated by Pelletier(12). He did not use standards, but calculations from his graph of ascorbic acid depletion indicate that the activity we found in facial vein blood was approximately the same as that obtained by the cavernous sinus puncture method of MacFarland, Clegg and Ganong (15). The facial vein approach offers simplicity and is not complicated by possible internal hemorrhage, impalement of the pituitary or arterial contamination of samples by puncture of the intracavernous rete mirable.

In conclusion, the present study demonstrates a method for obtaining venous blood draining the hypothalamus and the pituitary gland. Further, LH, as measured by the ovarian ascorbic acid depletion method, is detectable in samples drawn by these methods. It is hoped that further studies will be able to demonstrate other pituitary hormones in such samples.

Summary. A method is outlined whereby the great cerebral vein (of Galen) can be cannulated *via* an intradural sinus approach through the superior sagittal sinus. This allows for collection of venous blood draining the hypothalamic area of the brain. Also, a method for obtaining cavernous sinus blood *via* the facial vein is described. Assays of plasma obtained from these vessels revealed that they contained more LH (OAAD activity) than samples obtained from a leg vein.

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Effects of Serum Uric Acid and Parotid Flow Rate on Concentration and Secretion Rate of Uric Acid in Parotid Fluid.* (31097)

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A prime objective of this laboratory is the development of additional capabilities in biomedical monitoring by the use of parotid fluid. Such endeavor is intimately related to the use of this fluid in the diagnosis of disease, an area in which a measure of success has been attained(1-3). Uric acid measurements, generally carried out on serum or urine, are extremely important in a diagnostic sense. Increased levels of this end product of purine metabolism are seen in association with increased nitrogen retention and increased urea, creatinine, and other nonprotein nitrogen constituents of blood.

The present study is a primary effort in evaluating parotid fluid uric acid as a possible diagnostic measurement. It explores the relationship between uric acid values in serum and parotid fluid and determines the effect of flow rate on parotid fluid uric acid. The flow rate level is very low and the range is narrow since no exogenous stimulants were employed. The correlation coefficients are of the between-subject type since only one saliva sample was provided by each subject.

Materials and methods. Subjects were 508 males between the ages of 17 and 22 years. Each was found fully qualified for unrestricted military duty by recent medical examination. Times of arising and retiring, physical exertion, intake of food and liquids, as well as environmental and emotional exposure were very similar for all participants. This was a barracks-dwelling group of USAF enlistees with all of the homogeneity that this implies. Each subject had resided in this environment and ingested the basic military ration for at least 3 weeks prior to participation.

Subjects fasted overnight and a 2-hour parotid fluid collection period began at approximately 7:30 a.m. No exogenous stimulants were employed, this being an attempt to obtain, as nearly as possible, the resting secretion of the gland. Subjects were seated comfortably in a quiet portion of the temperature-controlled laboratory and a parotid sampling device(4) was placed over the right Stensen's duct with an absolute minimum of manipulation. Extraneous interferences were prohibited and close supervision assured that each subject remained awake and alert. Parotid saliva was collected in graduated tubes and volume was read to the nearest 0.05 ml. A single venous blood sample was drawn from each participant at approximately 9:30 a.m.

Uric acid content of both parotid fluid and serum was measured on the Technicon AutoAnalyzer by a sodium cyanide-phosphotungstic acid procedure in which the blue color of the reaction is read at 660 mμ in a 15 mm light path. Such a procedure includes in serum approximately 10% chromogen that cannot be acted upon by uricase.

Results. The relationship between serum and parotid fluid uric acid concentrations is shown in Fig. 1. For the group of subjects with the lowest serum uric acid level (less than 4.01 mg/100 ml) the parotid fluid con-

* The opinions expressed in this paper are those of the author and do not necessarily reflect official USAF policy.

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