this activity (Table II). However, some data suggest that urinary blood group substance is either degraded or of smaller molecular size than salivary blood group substance(2,3) and such altered molecules may conceivably have altered activities.

Summary. The blood group activity in human urine has been investigated by means of inhibition of hemagglutination and the results compared to the activity of saliva. Blood Group A and B activity was the same when measured in saliva or urine, although a few urine specimens showed non-specific inhibition unless dialyzed. *Ulex* extract inhibiting activity was present in saliva specimens of secretors from all blood groups, whereas in urine, this activity could not be demonstrated except in specimens obtained from Group O individuals. *Ulex* extract is not useful, therefore, as a reagent for determining the secretor status of an individual when urine is the source of antigen.

We wish to acknowledge the valuable technical assistance of Mr. Leroy Samuels.

1. Race, R. R., Sanger, R., *Blood Groups in Man,* 3rd Ed., Charles C Thomas & Co., Springfield, Ill., 1959.

2. Kabat, E. A., *Blood Group Substances,* Academic Press Inc., New York, 1956.

3. King, J. S., Fielden, M. L., Boyce, W. H., *Arch. Biochem. & Biophys.,* 1960, v90, 12.

Received May 24, 1962. P.S.E.B.M., 1962, v110.

Pituitary Gonadotrophic and Growth Hormone Levels in Sheep Plasma.* (27624)

E. F. ELLINGTON,† A. N. CONTOPOULOS AND M. T. CLEGG

Department of Animal Husbandry, University of California, Davis, and Department of Anatomy, San Francisco Medical Center, University of California

Considerable effort has been expended on the development of methods for quantitation of pituitary hormone levels in blood. Although there have been some studies reporting gonadotrophic activity in the blood of small non-pregnant laboratory animals such as the gonadectomized rat(1,2,3,4), the estrous rabbit(5), and the mouse(6), the extensive literature, recently reviewed(7,8), is concerned predominantly with investigations concerning levels of these hormones in human blood.

With regard to blood gonadotrophic activity in the larger non-pregnant mammalian species, very little information is available; such activity has been demonstrated in the estrous pig(9), and in the non-pregnant mare (10). Rimington and Rowlands(11) could not confirm the presence of hormone in the blood of the non-pregnant mare; and Santolucito(12) could not detect circulating gonadotrophic hormones in sheep blood during various stages of the estrous cycle. Reports demonstrating the presence of growth hormone activity in the blood of non-human mammalian species are similarly rare. Gemzell *et al.*(13) reported values of 0.57 μg of growth hormone per ml in a calf, whereas Trenkle *et al.*(14) found values ranging from 5 to 74 μg of growth hormone per 100 ml of serum from non-pregnant and non-lactating sexually mature cows.

Recently a simple technic has been developed(15) for collection of blood from the cavernous sinus of sheep. Inasmuch as the cavernous sinus is one of the main avenues for venous drainage of the pituitary, the opportunity to detect pituitary hormones in blood is considerably enhanced; the concentration of these substances should be higher in blood obtained from the cavernous sinus. The following study was undertaken to compare gonadotrophic and growth hormone activity in blood plasma from the cavernous sinus as compared with levels in blood obtained from jugular or recurrent tarsal veins.

Materials and methods. Samples of plasma

* Supported in part by U.S.P.H.S. grants and grant-in-aid from Syntex Animal Products.

† NIH Predoctoral Fellow.

were prepared from blood obtained from 20 sheep (mixed breed) varying in age from 2 to 4 years. All animals had been orchidectomized at 2 to 6 weeks of age.

Samples of blood from the cavernous sinus were obtained from unanesthetized animals using the cavernous sinus technic(15). For each collection period, the volume of blood obtained from a single animal ranged from 50 to 150 ml. Comparable volumes of blood from the recurrent tarsal or jugular veins were collected at the same time. All blood samples were placed in heparinized tubes and immediately cooled in an ice bath. Within one hour the blood was centrifuged under refrigeration and the plasma aspirated, frozen, lyophilized and stored.

Assays were conducted in immature female rats, hypophysectomized at 26-28 days of age, used 2 weeks postoperatively. The dried pooled plasma was reconstituted with sterile distilled water in such a way that each assay animal received a total dose equivalent to 5, 10, 15, 20, 25, or 30 ml of original plasma in 4 days. The actual injection volume of the reconstituted plasma was one ml daily over the 4-day period. At autopsy the uterus and ovaries were removed from each animal and weighed; the ovaries were fixed in Bouin's solution, sectioned and stained with hematoxylin and eosin. The right tibia was removed, sectioned and stained with silver nitrate; the width of the proximal tibial epiphyseal plate was used as a measure of growth hormone activity(3,13). Completeness of hypophysectomy of the assay animals was checked by examination of the sella turcica. Data from incompletely hypophysectomized animals were discarded. Uninjected hypophysectomized rats were included in each assay series. The FSH potency of the plasma was expressed as the minimum amount of injected material necessary to initiate follicular growth in ovaries of recipient rats(16). The increase in size of the ovarian follicles was established by measuring with an ocular micrometer. An increase of 75 μ over the average follicular size of 350 μ present in the uninjected controls, was considered as a positive response. ICSH potency was determined by the minimal dose necessary to initiate repair of ovarian interstitial tissue(17).

Results and discussion. Table I shows the results obtained from bioassay of the plasma. Gonadotrophic and growth hormone-like activity was not found in significant amounts in the plasma obtained from the peripheral veins. Evidence of increased levels of these hormones was obtained in the cavernous sinus plasma as compared with peripheral plasma. The injection of 20 ml equivalent of cavernous sinus plasma over a period of 4

TABLE I. Growth and Gonadotrophic Response of Immature Hypophysectomized Rats Injected with Castrate Male Sheep Plasma.

				Ovaries					Uterus
					FSH response		ICSH response		
Material inj.	Total plasma dose, ml	No. of rats	Epiphyseal tibial cartilage width, μ	Wt, mg	Pos.	Neg.	Pos.	Neg.	Wt, mg
Cavernous sinus (venous) plasma	5	6	181 ± 11*	11 ± 1	0	6	0	6	27 ± 1
	10	12	190 ± 8	12 ± 1	1	11	0	12	31 ± 2
	15	11	178 ± 10	14 ± 1	3	8	0	11	33 ± 1
	20	16	204 ± 12	16 ± 2	7	9	6	10	42 ± 5
	25	5	187 ± 10	24 ± 6	4	1	3	2	74 ±20
Systemic (venous) plasma	5	4	156 ± 13	9 ± 1	0	4	0	4	33 ± 4
	10	10	161 ± 9	10 ± 1	0	10	0	10	29 ± 2
	15	12	164 ± 6	10 ± 1	0	12	0	12	27 ± 2
	20	17	172 ± 7	10 ± 1	2	15	0	17	33 ± 3
	25	8	170 ± 9	10 ± 1	0	8	0	8	32 ± 2
	30	7	172 ± 14	14 ± 1	0	7	0	7	31 ± 1
Uninj. controls	0	15	144 ± 6	8 ± .5	0	15	0	15	23 ± 1

Criterion of positive response for FSH; presence of ovarian follicles with avg diameter of 425 μ or more; for ICSH: repair of ovarian interstitial tissue.

* Mean ± S.E.M.

days into recipient hypophysectomized immature female rats resulted in a significant increase in ovarian and uterine weight. This observation was supported by the results of histological examination of the ovaries. The minimum effective dose (MED) for FSH or amount of material necessary to produce a positive response in 50% of the animals was approximately 20 ml of plasma per day, whereas the MED for ICSH was somewhat higher, between 20 and 25 ml of plasma per day. Thus the FSH/ICSH ratio in plasma was slightly greater than one.

Although injection of 25 ml of peripheral plasma resulted in a slight increase in ovarian and uterine weight, a total dose of 30 ml of systemic plasma failed to cause stimulation of either follicular growth or interstitial cell repair. The relationship between amount of cavernous sinus plasma injected and increase in ovarian and uterine weight can be noted in Table I. Injections of plasma from cavernous sinus blood caused a significant increase in the width of the epiphyseal cartilage plate as compared with untreated hypophysectomized controls. Some increase in epiphyseal cartilage width was obtained at all dose levels of cavernous sinus or systemic plasma. Greatest stimulation occurred when a total dose of 20 ml of cavernous sinus plasma was injected. In all but one instance, systemic plasma resulted in a consistently smaller increase of the epiphyseal cartilage plate width than for a comparable dose of cavernous sinus plasma. A dose-response relationship between graded doses of cavernous sinus or systemic plasma and the width of the epiphyseal cartilage plate is not apparent.

These results clearly demonstrate the presence of gonadotrophic hormone activity in cavernous sinus plasma of castrated male sheep. Although equivalent or larger doses of peripheral plasma resulted occasionally in increases of ovarian and uterine weights, the failure to demonstrate a graded response with increasing amounts of such plasma (and without histological changes in the ovaries) would suggest that these small differences were not necessarily related to gonadotrophic hormone activity. The systemic plasma derived from castrated male sheep failed to stimulate consistently follicular growth in the recipient hypophysectomized animals, and no evidence of ICSH activity was apparent. A total of 15 to 20 ml of cavernous sinus plasma, however, caused an ovarian and uterine weight stimulation accompanied by active follicular development, as judged by the appearance of medium to large follicles; more importantly a total dose of 20 to 25 ml of cavernous sinus plasma produced interstitial cell repair. The finding that ICSH and FSH were detected in the cavernous sinus blood and not in the peripheral blood might be explained on the basis of dilution of the hormone level near the site of production, or to a peripheral utilization of these hormones. This finding is in contrast to previous demonstration of an increase in follicle-stimulating activity in plasma of gonadectomized rats where stimulation of the interstitial cells, however, was not apparent(3,4). Since plasma from gonadectomized animals was not compared with that from intact male sheep, it is impossible to say whether an increased FSH and ICSH activity resulted following the removal of the testes. This is presently being investigated.

The typical straight-line relationship between the cartilage plate response and the logarithm of the dose of growth hormone was not obtained in this experiment as has been reported in other cases(3,18). The fact, however, that 20 ml of cavernous sinus plasma produced an increase in cartilage width (in excess of 50 μ) over that of controls can only be accounted for by the presence of growth hormone-like activity. The lack of greater response with larger doses of plasma might be explained by the presence of other hormones, like ACTH and gonadotrophic hormones which tend to depress the normal response of tibial epiphyseal width to growth hormone. Toxic effects of large doses of plasma also cannot be ruled out as contributing to these effects.

This study, together with an earlier report by McFarland, Clegg, and Ganong(15) clearly demonstrates that blood obtained from the cavernous sinus of sheep contains a sufficiently high concentration of pituitary hormones to make detection of gonadotrophic

and growth hormone possible by present bioassay technics.

Summary. FSH and ICSH and growth hormone-like activity were demonstrated in the cavernous sinus plasma of gonadectomized male sheep, and in apparent higher concentration than found in peripheral plasma.

1. Emery, F. E., *Am. J. Physiol.,* 1932, v101, 246.
2. Hellbaum, A. A., Greep, R. O., *Endocrinology,* 1943, v32, 33.
3. Contopoulos, A. N., Simpson, M. E., Koneff, A. A., *ibid.,* 1958, v63, 642.
4. Cozens, D. A., Nelson, M. E., PROC. SOC. EXP. BIOL. & MED., 1958, v98, 617.
5. Soliman, F. A., Ghanem, Y. S., *Nature,* 1956, v178, 745.
6. Martins, T., *Compt. rend. soc. biol.,* 1929, v102, 605.
7. Antoniades, H. N., *Hormones in Human Plasma,* Little, Brown & Co., Boston, Mass., 1960.
8. Gray, C. H., Bacharach, A. L., *Hormones in Blood,* Academic Press, New York, 1961.
9. Lozinski, E., Holden, G. W., Macallum, E. N., *Rev. Canad. Biol.,* 1942, v1, 677.
10. Cole, H. H., Goss, H., *Am. J. Physiol.,* 1939, v127, 702.
11. Rimington, C., Rowlands, I. W., *Biochem. J.,* 1944, v38, 54.
12. Santolucito, J. A., Ph.D. Dissertation, Univ. of California, Davis, 1957.
13. Gemzell, C. A., Heijkenskjold, F., Strom, L., *J. Clin. Endocrinol. & Metab.,* 1955, v15, 537.
14. Trenkle, A., Burroughs, W., Melampy, R. M., Quinn, L. Y., *J. Animal Sci.,* 1960, v19, 1338.
15. McFarland, L. Z., Clegg, M. T., Ganong, W. F., PROC. SOC. EXP. BIOL. & MED., 1960, v103, 538.
16. Evans, H. M., Simpson, M. E., Tolksdorf, S., Jensen, H., *Endocrinology,* 1939, v25, 259.
17. Simpson, M. E., Li, C. H., Evans, H. M., *ibid.,* 1942, v30, 977.
18. Contopoulos, A. N., Simpson, M. E., *ibid.,* 1957, v61, 765.

Received May 28, 1962. P.S.E.B.M., 1962, v110.

Attempts to Enhance Infectivity of Adenovirus Type 8 for Cell Cultures.* (27625)

L. HANNA AND E. JAWETZ
Department of Microbiology, University of California Medical Center, San Francisco

Adenovirus type 8 shares many characteristics (*e.g.*, size, morphology, ether-resistance, cytopathogenicity, complement-fixing group antigen) with other adenoviruses. However, it differs from other types in producing a specific and unique eye disease in man. Adenovirus type 8 is the principal and perhaps the sole infectious agent capable of regularly producing typical epidemic keratoconjunctivitis (EKC) in man after natural or experimental infection(1).

Isolates of type 8 from many parts of the world, studied in different laboratories, have shown uniformly low infective titers for many different cell lines and for some primary cells in culture. It has been suggested that this uniformly low infectivity is a function of the exceedingly small proportion of fully infective particles in the virus population grown in tissue culture(2). Much evidence indicates that virus populations growing in the adult human eye may also have only a minute proportion of fully infective particles. This small amount of infective virus released accounts for the low spontaneous communicability of the infection and the importance of iatrogenic spread(3).

The question arises whether propagation in cell culture at selective conditions might raise the infective titer of adenovirus type 8. To that end we have performed 2 series of experiments reported here. In one we evaluated the effect of different incubation temperatures, in the other the effect of inoculum size, comparing limiting dilution to undiluted tissue culture.

Materials and methods. Viruses. Two strains of adenovirus type 8 were employed. One was the prototype strain (Trim) isolated in this laboratory(4), the other a Japanese

* Supported by grants from U.S.P.H.S. and Burroughs Wellcome Fund.