Siliceous filters have a negative charge and hold the basic dye particles which have a positive charge. When the filtering surface is covered with the "wetting agent" this prevents the siliceous surface from holding the positively charged dye particles.

This hypothesis is borne out by the following experiment. A clean glass rod was immersed in the dye solution in distilled water and allowed to stand over night. The rod will be found stained blue and the dye can not be washed off with water. Another glass rod is immersed in a dye solution containing 1:2000 castor oil soap, or a similar solution of sodium taurocholate, or a solution of sodium lauryl sulfate of similar concentration. The rods are allowed to stand in these solutions over night, yet when they are removed and washed off with water, they are unstained.

The "wetting agent" has insulated the surface of the glass rod, which has a negative charge, so that it can not hold the positively charged dye particles.

It is a pleasing duty to acknowledge my thanks to the staff of the National Institute of Health for the facilities placed at my disposal.

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Effect of Certain Endocrines on the Excretion of an Antidiuretic Substance.

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The activity of the posterior pituitary has been investigated with renewed interest during the past few years. It has been shown² recently and confirmed^{1, 4, 5} that an antidiuretic substance is excreted in the urine following dehydration of animals. Gilman and Goodman believe this substance is of pituitary origin since it is absent in the urine of animals dehydrated after hypophysectomy.

The relationship of the antidiuretic activity of the posterior pituitary to the other members of the endocrine system is still not clear. It has been reported that it is related to adrenal cortical function only

¹ Boylston, G. A., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 644. ² Gilman, A., and Goodman, L. S., J. Physiol., 1937, **90**, 113.

⁴ Ingram, W. R., Ladd, L., and Benbow, J. T., Proc. Am. Physiol. Soc., 1938, 107. ⁵ Martin, S. J., Herrlich, H. C., and Fazekas, J. F., Am. J. Physiol., 1939,

¹²⁷, 51.

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in so far as they are both concerned with water metabolism.⁵ In this investigation, an attempt has been made to determine the effect of chronic daily injections of certain sex hormones on the ability of the posterior pituitary to excrete an antidiuretic substance during dehydration.

A series of 84 adult albino and piebald rats were used in this study, 4 of which were females and were hypophysectomized, the remainder were males and were divided into groups for injections of endocrine preparations. The products employed* were oil solutions of theelin, testosterone propionate and progesterone and the gonadotropic principle of human pregnancy urine (Antuitrin S). All injections were made subcutaneously once or twice daily in the amount and for the duration noted in Table I. The concentrations were adjusted so that the daily injected amounts varied only from 0.1 cc to 0.3 cc. At the end of the experimental period, the hypophyses of 14 theelin- and 12 testosterone-treated rats were assayed for their gonadotropic activity by the method of intramuscular implantation into immature female rats.⁶

Throughout this study, the rats were kept in groups of 4 in cages constructed to facilitate urine collections. Twenty-four-hour urine samples were collected after periods of (1) free access to water, (2) 24-hour dehydration, and (3) 24-hour dehydration beginning 1-3 days after the injections of the endocrine preparations were stopped. The samples were bioassayed for the presence of an antidiuretic principle according to the technic modified by Gilman and Goodman. As in previous studies, the *index of diuresis* was regarded as the number of minutes required for a group of 4 hydrated rats to excrete one-half the amount of water given by stomach tube after a subcutaneous injection of the dialyzed, concentrated 24-hour urine sample. All rats were given a standard diet and the usual laboratory care.

Assays were also made on similar daily urine collections obtained from the 4 female rats intermittently on different days, during a 12-day period after hypophysectomy. Successful removal of the pituitary was shown by the post-operative absence of oestrus with the aid of daily vaginal smears as well as by the absence of pituitary tissue upon careful autopsy examination.

Results. The assays of urine obtained from all rats given free access to water showed an average index of diuresis of 109 minutes.

^{*} We wish to express our thanks for the generous supply of theelin and Antuitrin S from Dr. Oliver Kamm, Parke, Davis & Co., and of testosterone propionate and progesterone from Dr. Erwin Schwenk, Schering Corp.

⁶ Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., *Endo.*, 1932, **16**, 655.

Procedure	No. of animals	Daily dos- age per rat	No. of days of injections	Total No. of assays	Index of Diuresis avg in min	Remarks
Control	84			21	166 (147-201)	Before injections
Injections of:					X y	U
Theelin	24	200 i.u.	26	12	180 (148-255))
Progesterone	12	2 mg	10	13	157 (134-209)	After cessation
Testosterone propionate	24	$1 \mathrm{mg}$	24	12	154 (136-195)	of
Antuitrin S	12	20 i.u.	23	10	164 (138-210)]

 TABLE I.

 Index of Diuresis Following Dehydration of Adult Rats After Chronic Injections of Endocrine Preparations.

This value was normal and indicated the absence of an antidiuretic substance. When the animals were dehydrated for 24 hours, the average index was 166 minutes, showing that an antidiuretic principle was present in the urine. In 8 tests on the dehydrated hypophysectomized rats, the index of diuresis was 104.5 minutes (range 92-116 minutes) and was therefore within normal values.

The effect of chronic daily injections of large doses of various endocrine preparations on the excretion of an antidiuretic substance after dehydration is presented in Table I. A total of 58 bioassays were performed. All rats showed a normal appetite throughout this study. It will be noted that the group averages for the indices of diuresis (154-180 minutes) cover a fairly wide range. However, in each determination, except one not recorded in the table, the index was significantly greater than the normal control value and hence definitely indicated the presence of an antidiuretic substance in the urine. The ability of the pituitary to respond to the stimulus of dehydration was not diminished by chronic injections of the endocrines used. That theelin and testosterone injections had some effect on the pituitary was shown by a decrease in gonad stimulating power of the anterior lobe of 43 and 35% respectively as determined by differences in the ovarian weights of immature rats.

It appears fairly well established that an antidiuretic substance is excreted in the urine as a result of dehydration whether this be from simple water deprivation^{1, 2, 4} or hypoadrenia.⁵ Whether such a substance is solely of pituitary origin may remain open to question until adequate chemical methods of identification replace the bioassay procedure now used. However, the significant fact still remains that an antidiuretic principle is not present in the urine of dehydrated

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animals after hypophysectomy. This finding of Gilman and Goodman² is confirmed by our results.

It has been previously shown that large doses or prolonged injections of theelin⁶ or of testosterone propionate^{3, 7} depress the gonadstimulating power of the anterior hypophysis. While we have noted this, our results also show that these preparations do not affect the posterior lobe in its ability to secrete an antidiuretic substance following dehydration. Furthermore, progesterone and antuitrin S similarly have no effect. In one respect, therefore, the sex hormones are not directly related to posterior pituitary activity. Indirect relationships, of course, may be possible.

Conclusions. Prolonged injections of large doses of theelin, progesterone, testosterone propionate and antuitrin S do not alter the ability of dehydrated rats to excrete an antidiuretic substance. Since this substance appears to be of hypophyseal origin, these sex hormones have no direct relationship to this phase of posterior pituitary activity.

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Effects of a Digested Pituitary Extract on Reproductive Tract of Hypophysectomized Adult Male Rats.

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The work of McShan and Meyer¹ and Chen and van Dyke² has shown that the luteinizing ability of pituitary gonadotropic extracts can be largely destroyed by tryptic digestion. These treated preparations produced remarkably specific gametogenic effects in normal and hypophysectomized immature female and male rats.

Greep and Fevold³ have shown that the hypophyseal fraction LH acts on the interstitial cells of hypophysectomized adult male rats causing the secretion of male hormone, whereas the FSH preparation will sustain or repair the gametogenic processes without stimulating the testes to endocrine function. However, with dosages of FSH in

³ Hamilton, J. B., and Wolfe, J. M., Endo., 1938, 22, 360.

⁷ Moore, C. R., and Price, D., Endo., 1937, 21, 313.

¹ McShan, W. H., and Meyer, R. K., J. Biol. Chem., 1938, 126, 361.

² Chen, G., and van Dyke, H. B., PROC. Soc. EXP. BIOL. AND MED., 1939, 40, 172.

³ Greep, R. O., and Fevold, H. L., Endocrinology, 1937, 21, 611.