

with 1 ml of M/100 copper sulphate and 8 ml of 0.5 N sodium hydroxide. To this was then added dropwise with whirling 3 ml of the $\frac{1}{3}$ dilution of Folin's³ phenol reagent. The solutions were then read after 5-10 minutes against 0.15 mg tyrosine treated in a similar manner.

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Use of Anterior Lobe of Prostate Gland in the Assay of Metakentrin.

R. O. GREEP, H. B. VAN DYKE AND BACON F. CHOW.

From the Division of Pharmacology, the Squibb Institute for Medical Research, New Brunswick, N.J.

We believe that the recent isolation of a homogeneous protein, from pituitary tissue, which has the property of causing the formation of corpora lutea from preformed graafian follicles and of stimulating ovarian and testicular interstitial cells in rats establishes the individuality of a gonadotrophin with some but not all the properties of luteinizing hormone as originally recognized by Fevold, Hisaw and their coworkers. During the course of our purification procedures, we have utilized the weight increase of the anterior lobe of the prostate gland in the assay of the interstitial cell-stimulating hormone^{1, 2, 3}—metakentrin.⁴ The principal objection to this procedure lay in the possibility that the substance stimulating the secretion of androgen by the interstitial cells of the male gonad was not identical with the substance causing formation of corpora lutea. Following the isolation of metakentrin¹ we have determined that under proper conditions all these biological effects are manifestations of this pure preparation. We are prompted, therefore, to record our experience and technic in assaying this hormone by the ventral prostate method.

Our standard procedure is as follows: The animals used are hypophysectomized immature male rats. The operation is per-

³ Folin, O., and Ciocalteu, V., *J. Biol. Chem.*, 1927, **73**, 627.

¹ Shedlovsky, T., Rothen, A., Grep, R. O., van Dyke, H. B., and Chow, B. F., *Science*, 1940, **92**, 178.

² Chow, B. F., Grep, R. O., and van Dyke, H. B., *J. Endocrinol.*, 1939, **1**, 439.

³ Grep, R. O., Chow, B. F., and van Dyke, H. B., *J. Biol. Chem.*, 1940, **133**, 289.

⁴ Coffin, H. C., and van Dyke, H. B., *Science*, 1941, **93**, 61.

formed at 21-22 days of age, body weight being selected to fall between 35 and 47 g. Injections are started after a 2-day post-operative interval and are continued for 4 days with autopsy on the 5th day. One cubic centimeter of solution representing one-fourth of the total dose is administered subcutaneously once daily for 4 days. Removal of the anterior prostate free from surrounding connective tissue and fat can be easily accomplished; dissection of definitely atrophic glands is facilitated by the use of a dissecting binocular. The glands are placed in saline from which they are removed and weighed on a torsion balance (accuracy ± 0.02 mg) after excess of fluid has been quickly absorbed by filter paper.

Control organs were taken from untreated animals killed 6 days after hypophysectomy. The weights of the anterior prostate and the testes of such animals fall within close limits. For example, the mean and standard error of the weight of the anterior prostate of 57 control rats were 6.16 ± 0.118 mg. Similar data with respect to the pairs of testes of these animals were 109.6 ± 1.11 mg. Our experience indicates that only occasionally rather than routinely is it necessary to include a control group with other groups receiving hormone. A minimum of 5 animals per dose is satisfactory for routine tests but for critical assay, at least 10-20 animals per dose is desirable.

Fevold⁵ uses the hypertrophy of the seminal vesicles of normal immature male rats as a means of assaying luteinizing hormone. Chow, Grep, and van Dyke² were unable to produce gross stimulation of the seminal vesicles in hypophysectomized immature males after the injection of amounts of hormone causing great enlargement of the anterior prostate. The data in Table I show the marked response of the prostate as well as the virtual lack of response of the seminal vesicles of the hypophysectomized immature male rat to testicular secretion induced by a pituitary extract in which the gonadotrophins had not been fractionated. Many experiments were also performed in which the period of injection was 8 days instead of 4; these are recorded in Table I. A dose (75.0 mg equivalent of fresh gland) 6 times greater than was required to produce a significant enlargement of the anterior prostate failed to enlarge the seminal vesicles. Moreover, there was no correlation between dose of extract and response of seminal vesicles, whereas good correlation was found with respect to the anterior prostate. (Work reported from the laboratory of H. M. Evans likewise failed to demonstrate that changes in the seminal vesicles can be

⁵ Fevold, H. L., *Cold Spring Harbor Symposia*, 1937, **5**, 93.

TABLE I.
Effect of Unfractionated Gonad-stimulating Extract of Hog Pituitary on Testes
and Accessory Organs of Hypophysectomized Immature Rats.

No. rats	Treatment		Avg body wt, g	Mean and S. E. of wt of			
	Days	Total mg eq. fr. gl.		Testes, mg	Ant. prost., mg	Seminal vesicles,* mg	
17	—	—	38.4	102.4± 2.96	7.83±0.299	6.24±0.200	
17	4	12.5	34.3	135.2± 6.19	12.75±0.709	6.52±0.173	
17	4	25.0	40.1	191.5± 6.37	15.09±0.912	7.24±0.196	
17	4	75.0	38.3	219.5± 9.51	18.08±0.770	7.29±0.256	
17	4	150.0	38.3	245.4± 7.58	20.48±1.201	7.69±0.257	
17	—	—	40.3	81.1± 4.34	5.44±0.191	6.00±0.238	
15	8	12.5	43.0	125.7± 6.23	12.97±1.369	7.16±0.241	
16	8	25.0	42.1	132.7± 3.72	11.92±0.651	6.05±0.209	
15	8	25.0†	42.0	172.8± 8.88	15.29±1.077	6.81±0.177	
15	8	37.5	43.6	209.0±13.52	17.45±1.384	7.10±0.851	
16	8	50.0	41.6	212.5± 7.70	15.87±0.898	6.01±0.152	
16	8	75.0	42.5	252.4±10.71	20.54±1.409	6.37±0.347	
17	8	150.0	42.7	321.7± 9.92	22.86±1.165	7.42±0.211	
18	8	300.0	42.7	328.6±14.43	25.99±1.906	8.35±0.429	
15	8	600.0	40.8	338.6± 9.44	28.48±2.242	7.78±0.352	
17	8	1000.0	44.5	390.0±14.05	38.25±2.998	9.11±0.526	
16	8	3500.0	43.8	371.5±10.77	41.87±2.650	7.63±0.186	

*Including coagulatory glands.

†Intraperitoneally; all others subcutaneously.

satisfactorily used for the assay of interstitial cell-stimulating hormone.) Our attempts to produce a response in the seminal vesicles of normal immature males by this means have also met with poor success (Table II). The anterior prostate of normal rats undergoes hypertrophy but we cannot recommend its use in the assay of metakentrin; the amount of prostate growth seen in untreated control animals shows how seriously the presence of the test animal's own pituitary may complicate the assay.

Fevold⁶ has concluded that the stimulating effect of luteinizing

TABLE II.
Effect of Unfractionated Gonad-stimulating Extract of Hog Pituitary on Testes
and Accessory Organs of Normal Immature Rats. Injections Were Made Intra-
peritoneally.

No. rats	Treatment		Age, days	Necropsy: Mean wt of			
	Days	Total g eq. fr. gl.		Body, g	Testes, mg	Ant. prost., mg	Sem. Ves.,* mg
11	—	—	21	38.5	175.1	24.10	8.30
11	—	—	25	45.8	309.0	29.00	8.00
8	4	5	25	44.4	405.1	47.85	10.12

*Including coagulatory glands.

⁶ Fevold, H. L., *J. Biol. Chem.*, 1939, **128**, 83.

hormone, derived from sheep pituitaries, on the seminal vesicles of normal or hypophysectomized immature males is potentiated by the administration of follicle-stimulating hormone of similar origin. It is clearly possible to interpret this observation in several ways. We wish only to emphasize that the assay technic here recommended is specific for metakentrin and is not influenced by the simultaneous administration of biologically pure thylakentrin* (Table III).

TABLE III.
Lack of Effect of Thylakentrin (FSH) on Response of Prostate of Hypophysectomized Immature Rats Receiving Metakentrin (ICSH).
(Hormones were combined *in vitro*.)

No. rats	Dose in μg N		Mean wt	
	Metakentrin	Thylakentrin	Testes, mg	Ant. prost., mg
4	0	0	116.1	6.86
4	0	20	187.9	7.89
6	4	0	128.4	10.02
5	4	20	187.4	11.36
4	0	30	204.4	7.25
5	2	0	148.7	11.48
6	2.5	30	197.7	10.41
4	5	0	124.7	13.77
5	5	30	212.8	11.87
8	2	0	130.8	10.77
7	2	50	222.9	11.94

Evans, Korpi, Simpson, Pencharz and Wonder⁷ observed that the atrophic interstitial cells of hypophysectomized female rats were repaired by their ICSH fraction but not by other pituitary hormones. Their earlier view that the interstitial cell-stimulating and luteinizing hormones are not identical has been withdrawn^{8, 9} and the repair of the interstitial cells of hypophysectomized female rats is now used by them in the assay of luteinizing hormone. The relative sensitivity of this technic as compared with ours has not been accurately determined. Our data, however, indicate that the ovarian interstitial

* This name was introduced by Coffin and van Dyke⁴ to refer to pure-follicle-stimulating hormone whose action appears to be solely to stimulate follicle growth unaccompanied by estrogen secretion unless metakentrin also is administered.

⁷ Evans, H. M., Korpi, K., Simpson, M. E., Pencharz, R. I., and Wonder, D. H., *Univ. Calif. Pub. in Anat.*, 1936, **1**, 255.

⁸ Jensen, H., Simpson, M. E., Tolksdorf, S., and Evans, H. M., *Endocrinology*, 1939, **25**, 57.

⁹ Evans, H. M., Simpson, M. E., Tolksdorf, S., and Jensen, H., *Endocrinology*, 1939, **25**, 529.

cells, judged by alterations in their histology, are somewhat less sensitive toward metakentrin stimulation than are the interstitial cells of the testes judged by the secondary effect of secreted androgen on the anterior prostate.

Fig. 1 summarizes graphically our experience with various doses of chemically pure metakentrin assayed by the method described in this report. Each point represents an average of 7.4 animals. Corresponding points such as open circles, crosses, etc., represent assays on one preparation carried out simultaneously at 2-3 dose-levels. The formula for the straight line was calculated by the method of least squares.

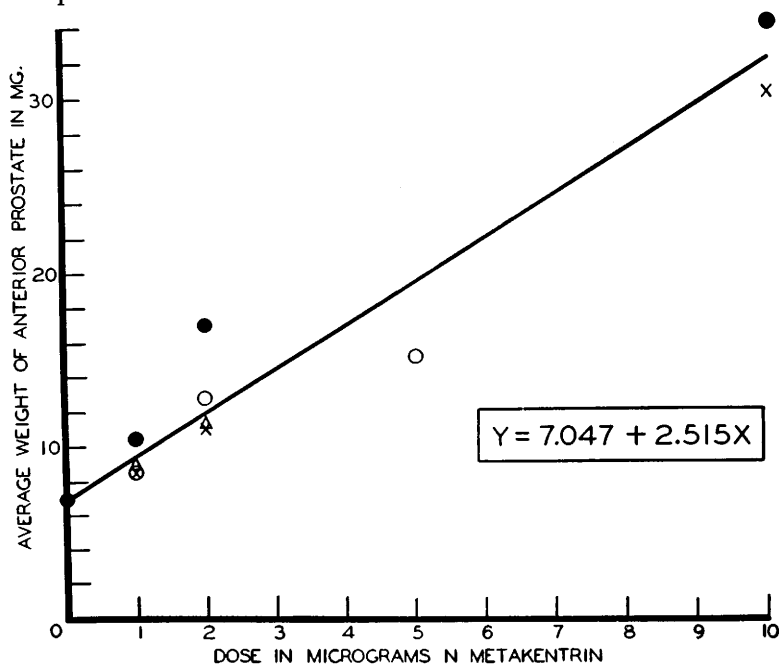


FIG. 1.

Dose-response curve of anterior prostate of hypophysectomized immature rats receiving total doses of 1-10 µg of N of pure metakentrin of which one-fourth was administered daily for 4 days. See also text.

The chief advantages in using the anterior prostate in the assay of metakentrin are: 1. The assay can be read objectively. 2. The assay can be placed on a quantitative basis. 3. No histological preparations or evaluations are required. 4. The presence of thylakentrin does not influence the assay. The chief disadvantages are: 1. The assay is not qualitative in that it does not show whether or not the preparation is free of thylakentrin. This must be determined by the use of hypophysectomized female rats unless the extract is free from

metakentrin; in such a case, male rats can be used and thylakentrin causes only testicular hypertrophy. 2. Prostatic enlargement being an indirect response mediated through the testes is subject to greater variability than would be expected were the effect direct as when prostate stimulation is produced by injected androgen.

Summary. In the assay of pituitary gonadotrophins, the enlargement of the anterior prostate of hypophysectomized immature male rats not only may be used as a specific test for metakentrin (ICSH), but also may be employed satisfactorily in making a quantitative assay of this substance.

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Deposition of Melanin in Sparrow Bill Following Local Action of Testosterone Propionate in Alcoholic Solution.*

ARTHUR KIRSCHBAUM AND CARROLL A. PFEIFFER. (Introduced by Edgar Allen.)

From the Department of Anatomy and the Adolescence Study Unit, Yale University School of Medicine, New Haven, Conn.

The bill of the English sparrow is very sensitive to the action of androgens.^{1, 2, 3} Intramuscular injections of androgens dissolved in oil have been shown to induce pigmentation of the bill in the castrate male, or in sexually inactive birds of either sex.^{1, 2, 3} During the breeding season the bill of the male is jet black. As the testis enlarges (during the winter months) previous to mating activity the bill changes progressively from the yellow-color of the juvenal or the sexually inactive adult to the deep black of the sexually active male.^{2, 3, 4} The bill of the female also becomes appreciably darker during the egg-laying season; this response is due to secretion of ovarian androgen and not to estrogenic hormone of the ovary.⁵

The demonstration of the local action of sex hormones indicates

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¹ Keek, W. N., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1140.

² Keek, W. N., *J. Exp. Zool.*, 1934, **67**, 315.

³ Witschi, E., *Proc. Soc. Exp. Biol. and Med.*, 1936, **33**, 484.

⁴ Kirschbaum, A., and Ringeon, A. R., *Anat. Rec.*, 1936, **64**, 453.

⁵ Pfeiffer, C. A., and Kirschbaum, A., *Yale J. Biol. and Med.*, 1941, **13**, 315.