GONADOTROPHIC HORMONE:

PROTECTION AGAINST INACTIVATION

by

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INTRODUCTION

Gonadotrophic hormones stimulate gonadal growth, maturation and secretion. The simultaneous administration of certain substances modifies the action of gonadotrophins. These substances have been classified according to their mode of action, as (1) synergists and (2) augmentors.

Synergists are themselves gonadotrophic substances which exert their modifying influence at the end-organ --the gonad. Synergism occurs whether the synergist is injected at the same site, or at a site distant from the gonadotrophin injection. It is exemplified by the increase in ovarian response which results from the simultaneous administration of chorionic gonadotrophin and pituitary gonadotrophin(1)

Augmentors exert their modifying influence by delaying absorption of gonadotrophins from the site of injection(2). They are inert if injected at a site different than the gonadotrophin(3,4). They include such chemically diverse materials as whole blood(5), aluminum hydroxide(6) and egg white(7).

It is the purpose of this communication to demonstrate a third type of gonadotrophin enhancing mechanism which operates <u>in vitro</u> in contrast to augmentors and synergists which exert their action <u>in vivo.</u> The mechanism involves protecting against the <u>in vitro</u> inactivation of gonadotrophins by certain materials named "protectors."

MATERIALS AND METHODS

Twenty-two to twenty-four day-old Sprague-Dawley female rats were used to assay the gonadotrophins. One cc. of the materials to be assayed was injected twice daily for 3 days. The animals were

killed 24 hours after the last injection. Ovarian and uterine weights were taken as the assay end-points.

Gonadotrophins from four sources were investigated. (1) Adult male and female rat anterior pituitary glands served as the source of gonadotrophic hormone in the majority of experiments.. The donor rats were castrated 20 to 58 days prior to removal of the glands in order to increase gonadotrophin content. After decapitation, the anterior lobes were removed immediately, macerated by alternately drawing them into and expelling them from a syringe, and suspended in sufficient Locke's solution so that each 6 cc. (amount for one assay rat) contained the equivalent of 10 mg. of 20-day castrate pituitary tissue. (2) Lyophilized sheep anterior pituitary powder* was dissolved in Locke's solution so that each 6 cc. contained 0.25 mg. (3) Chorionic gonadotrophin powder was dissolved in Locke's solution so that each 6 cc. contained 10.0 mg. (4) Human male castrate urinary gonadotrophin, concentrated by the alcohol precipitation method of Heller and Chandler[8] was dissolved in Locke's solution so that each 6 cc. contained the hormone concentrated from a two-hour urine aliquot.

Procedures were conducted immediately after the solutions of gonadotrophins were prepared. These consisted of incubation at 37°C., shaking, and storage at 3°C. with and without the addition of one of the following substances: whole blood, testis slices, brain slices, egg white, aluminum hydroxide, prepuberal urine and ultrafilter concentrate of prepuberal urine. All materials were stored at 3°C. during

*Generously supplied by Mr. Frederic Penger of Armour and Company.

the three-day injection period. Solutions were shaken in 125 cc. Erlenmeyer flasks in a Kahn bacteriological shaker.

Whole blood was obtained from adult rats by intracardial puncture. Sufficient blood was added so that each 6 cc. of solution contained 0.33 cc. of whole blood. Rat testis slices were added to the solution so that each 6 cc. contained 500 mg. of sliced testis. Rat brain slices were likewise added so that each 6 cc. of solution contained 500 mg. of sliced brain. Egg white was added so that 6 cc. of solution contained 0.33 cc. egg white. Aluminum hydroxide, prepared according to Johnson and Bradley quoted by McShan and Meyer(6), was added so that each 6 cc. solution contained 100 mg. of aluminum hydroxide. In the experiment where prepuberal urine was used, the rat anterior pituitary glands were taken up in urine, instead of Locke's solution, so that each 6 cc. of urine contained 10 mg. rat anterior pituitary. In the experiment where ultrafilter concentrate of prepuberal urine was used, sufficient material was added so that each 6 cc. of solution contained the material concentrated from a 4-hour urine aliquot.

RESULTS

Inactivation of castrated rat pituitary gonadotrophin suspension. Control pituitary suspensions were kept at 3°C. for comparison with suspensions that were incubated at 37°C. or shaken at 3° and 37°C. The data of Table 1 show that shaking for 2.5 hours at 3°C. and incubation for 2.5 hours at 37°C. caused little if any inactivation of the gonadotrophin whereas shaking for 2.5 hours at 37°C. caused complete

inactivation. Reference to Table 2 offers additional evidence that shaking at 37°C. for 2.5 hours completely inactivates rat pituitary gonadotrophin. A total of five separate comparisons were made. Whereas suspensions stored at 3°C. showed demonstrable gonadotrophic potency in each assay, suspensions shaken at 37°C. for 2.5 hours elicited average ovarian weights ranging from 8.2 mg. to 13.4 mg. and no uterine stimulation in any assay. This may be compared to the uninjected control average ovarian weight of 10.0 mg. and no uterine stimulation. Thus it may be concluded that rat pituitary gonadotrophin is totally inactivated by shaking at 37°C. for 2.5 hours. (In all instances where average ovarian weights fell within the range of uninjected controls, no uterine stimulation was noted. In all instances where ovarian weights greater than the range of uninjected controls were encountered, maximal uterine stimulation was noted. This generalization applies to Tables 1 through 5).

Protection against inactivation of castrated rat pituitary suspensions was afforded by the addition of whole blood, rat testis slices, rat brain slices, egg white and aluminum hydroxide to the rat gonadotrophin before shaking at 37°C. The data of Table 2 show that the addition of whole blood afforded the best protection against inactivation, whereas testis and brain slices, egg white and aluminum hydroxide afforded less complete protection. Raw prepuberal urine and concentrates of such urine prepared by ultrafiltration offered no protection. None of the above materials, using the same amount of material per assay animal as added to 6 cc. of gonadotrophin, had any demonstrable gonadotrophic activity.

An alternate explanation for the detection of gonadotrophins in the suspensions after shaking at 37°C. in the presence of a foreign material is that the added material served as an augmentor. Thus inactivation might occur as usual but sufficient augmentation might

TABLE 1

Effect of shaking and temperature upon inactivation of rat pituitary gonadotrophin.

	Rat gonadotrophin 3°C.	Rat gonadotrophin Shaken 2.5 hours at 3°C.	Rat gonadotrophin 37°C. for 2.5 hours	Rat gonadotrophin Shaken 2.5 hours at 37°C.	No. of assay rats per procedure
Average ovarian weights mgs.	34.2	27.8	29.5	11.7	8

Average ovarian weight of 40 uninjected controls = 10.0 mg. range: 4.8 to 16.5. (This value was obtained from uninjected controls included in each series of assays conducted and applies to Tables 2, 3, 4 and 5 also).

TABLE 2

Protection against inactivation of rat pituitary gonadotrophin

Protector (Amount per 6 cc.)	Rat gonadotrophin 3°C. No protector	Rat gonadotrophin shaken 2.5 hours at 37°C. No protector	Rat gonadotrophin + protector shaken 2.5 hours at 37°C.	Rat gonadotrophin shaken 2.5 hours at 37°C. then add protector	Rat gonadotrophin + protector 3°C.	No. of assay rats per procedure
.33 cc. whole blood	46.1	10.3	44.4	8.5	66.3	6
500 Gms. sliced testis	54.5	11.7	38.7	8.5*	——	6
500 Gms. sliced brain	53.6	13.4	29.6	——	——	3
.33 cc. egg white	36.8*	10.6*	20.3	10.1	48.8	3
100 mg. $Al(OH)_3$			30.0	10.2	59.2	2
6 prepuberal urine	27.2	8.2	6.6	7.5	31.1	3
4 hour ultrafilter concentrate prepuberal urine			8.1	——	——	3

*3 rats per dose

Figures represent average ovarian weights of assay rats expressed in milligrams.

TABLE 3

Protection against inactivation of rat pituitary gonadotrophin during 3 days storage at 3°C.

Protector (Amount per 6 cc.)	Rat gonadotrophin 3°C. No protector	Rat gonadotrophin mixed with protector during 3-day injection period	Rat gonadotrophin + protector added at each injection	No. of assay rats per procedure
.33 cc. blood	34.2	48.3	32.5	8

Figures represent average ovarian weights of assay rats expressed in milligrams.

TABLE 4

Protection against inactivation of sheep pituitary gonadotrophins

Protector (Amount per 6 cc.)	Sheep gonadotrophin 3°C. No protector	Sheep gonadotrophin shaken 2.5 hours at 37°C. No protector	Sheep gonadotrophin + protector shaken 2.5 hours at 37°C.	Sheep gonadotrophin shaken 2.5 hours 37°C. then add protector	Sheep gonadotrophin + protector 3°C.	No. of assay rats per procedure
100.0 mg. $Al(OH)_3$	46.1	9.4	44.1	18.2	49.0	3

Figures represent average ovarian weights of assay rats expressed in milligrams.

TABLE 5

Failure of shaking at 37°C. to inactivate human urinary and chorionic gonadotrophins

Type of gonadotrophin	gonadotrophin 3°C.	gonadotrophin shaken 2.5 hours at 37°C.	gonadotrophin shaken 5 hours at 37°C.	No. assay rats per procedure
Human urinary gonadotrophin	45.7	44.8	40.2	3
Chorionic gonadotrophin	23.8	22.3	----	3

Figures represent average ovarian weights of assay rats expressed in milligrams.

take place to restore gonadotrophic potency. Reference to Table 2 indicates that this possibility was ruled out for the action of whole blood, testis slices, egg white and aluminum hydroxide by adding these substances after shaking at 37°C. had been completed. In no instance was gonadotrophic activity noted.

Adding whole blood, egg white and aluminum hydroxide to the control pituitary gonadotrophin suspensions kept at 3°C. resulted in an increase in gonadotrophic activity. This could be due to augmentation, synergism (assuming that the blood contains some gonadotrophic hormone), or if inactivation occurs at 3°C. over a 3-day period, it could be due to protection against such inactivation.

Inactivation at 3°C. for three days. The data of Table 3 show that addition of whole blood to a suspension of rat pituitary gonadotrophin caused an increased gonadotrophic response. If this increased response were due to augmentation or synergism, then the same order of response should be elicited whether the blood was in contact with the gonadotrophin for the 3-day injection period or in contact only at the time of injection. Therefore blood was added to an aliquot of the suspension at each injection. No increase in potency was noted (Table 3). It is therefore concluded that (1), inactivation occurs in 3 days at 3°C. and that (2), whole blood exerts its enhancing effect by protecting against inactivation and not by augmentation or synergism.

Protection against inactivation of sheep gonadotrophin. Sheep gonadotrophin was inactivated by shaking for 2.5 hours at 37°C. Inactivation was prevented by adding aluminum hydroxide before incubation but not by adding aluminum hydroxide after incubation

(Table 4).

Shaking for 2.5 hours at 37°C. failed to inactivate human urinary and chorionic gonadotrophin (Table 5). Even shaking for 5 hours at 37°C. failed to decrease the gonadotrophic potency of urinary extracts. Since both extracts are relatively crude preparations it may be that the original material contains a substance which already serves as a protector. However, a more plausible explanation, considering that we were unable to demonstrate any "protector" in urine or urine concentrate, is that these gonadotrophic hormones are more stable than gonadotrophins extracted from pituitary glands.

DISCUSSION

Inactivation: Pituitary gonadotrophic hormones, although comparatively stable in dry powder form, are notoriously unstable in aqueous solution. Rowlands[9] found no loss of potency when pituitary gonadotrophin powder was heated to 100°C. for one hour. However, heating an aqueous solution of hormone just to boiling caused complete loss of potency[10]. Solutions stored at room temperature for 9 days lost approximately 50% of their original potency[9]. In contrast, storage of sheep pituitary extracts at icebox temperatures for periods of months resulted in relatively little loss of gonadotrophic potency as compared with solutions that were stored for 4 days at icebox temperatures[9,10].

The methods of assay of gonadotrophic substances in general use involve a 3 to 5 day injection period during which the solutions to be assayed are stored at icebox temperature. We have been unable to

find any previous report concerning the degree of inactivation that occurs during this 3 to 5 day period of storage at icebox temperature. In the present study, partial inactivation of rat gonadotrophin during 3 days storage at 3°C. has been demonstrated by the addition of a substance (whole blood) capable of protecting the hormone from inactivation (Table 3). On the other hand, Rowlands (9) and Van Dyke and Wallen-Lawrence (10) observed relatively little loss of potency of sheep pituitary extract stored at icebox temperatures for 5 to 12 months, as compared to solutions stored at icebox temperatures for 4 days. Two possible explanations of this apparent incongruity can be made. (1) Their sheep pituitary extracts were more stable than the rat pituitary suspension used in this study. (2) The inactivation of pituitary gonadotrophin solutions stored at icebox temperatures occurs, for the most part, during the first few days of storage.

Chorionic gonadotrophin is likewise much more stable in dry powder form than in aqueous solution. Although heating the dry powder to 100°C. for three hours results in no loss in potency, heating an aqueous solution to 100°C. for five minutes results in almost complete inactivation(11). Although unstable in the aqueous state, chorionic gonadotrophin solutions appear to be more stable than aqueous pituitary gonadotrophin preparations(12). The observation that chorionic gonadotrophin was not inactivated by shaking at 37°C. is in accord with past experience that chorionic gonadotrophin is more stable than pituitary gonadotrophins.

Although it is well known that proteins are denatured by shaking(13) the extreme susceptibility of pituitary gonadotrophic hormones to shaking at 37°C. has not, to our knowledge, been previously demonstrated.

Denaturation by shaking involves the mechanism of surface denaturation, the function of shaking being to increase the formation of new surface. It is interesting to note that surface denaturation of urease results in loss (probably complete) of biological activity(14).

Synergism: Certain gonadotrophic hormones are capable of enhancing the effects of other gonadotrophins to a greater degree than can be explained by simply adding the effects of the two hormones when given separately. Leonard(1) found that a combination of chorionic gonadotrophin and pituitary gonadotrophin increased the weight of the assay rats' ovaries more than double that which could be explained by simply adding the effects of the two materials when given separately. He further observed that this synergistic action occurred whether the two materials were combined and injected at the same site or if the materials were kept separate and injected at separate sites.

Synergism has also been demonstrated for the pituitary gonadotrophins, follicle-stimulating hormone (FSH) and interstitial-cell-stimulating hormone (ICSH, also known as luteinizing hormone, LH). Fevold, Hisaw and Leonard(15) found that regardless of dosage, a purified preparation of ICSH was incapable of increasing the ovarian weights of immature female rats. However, when combined with FSH, ICSH was capable of enhancing the ovarian weight increasing action of FSH as much as five-fold. Furthermore, this synergistic action occurred if ICSH was administered alone for three days immediately followed by the injection of FSH for three days. This effect was

also demonstrated using hypophysectomized assay animals. They concluded that the synergistic effect of ICSH was a direct action on the ovary.

Augmentation: As mentioned beforehand, numerous materials that alone have no demonstrable gonadotrophic action are capable of augmenting the action of certain pituitary gonadotrophin preparations. In view of the great number of known "augmentors" it would appear highly improbable that each augmented by a different mechanism. Available evidence indicates that augmentation is, in each instance, due to delayed absorption from the site of injection. The end result is to bring about a more gradual release of hormone from the site of injection, thus simulating the probable manner in which the pituitary secretes the gonadotrophic hormone into the blood stream. The explanation of augmentation on such a basis rests on the assumption that a more gradual release of hormone into the blood stream is more effective in stimulating the gonads. It is therefore interesting to note that Meyer and McShan[16] found beef and sheep anterior pituitary gonadotrophic potency to be enhanced approximately ten-fold by increasing the number of injections from 2 to 24 daily.

Augmentation does not occur if augmentor and hormone are injected at different sites[3,4]. This in accord with the view that augmentation is due to delayed absorption from the site of injection. The possibility still remains, however, that augmentation is due to an *in vitro* reaction between augmentor and hormone. Evans, Geithamel, Hines and Koch[2] tested this possibility by mixing augmentor and hormone *in vitro* and injecting directly into the blood stream (intra-

cardially). Augmentation did not occur when injections were made intracardially whereas it did occur when injections were made subcutaneously. They further showed that adrenalin injected along with the hormone caused an increase in gonadotrophic potency. It would appear, therefore, that the augmentation of pituitary gonadotrophic hormones by a variety of non-specific materials can be adequately explained on the basis of delayed absorption of hormone from the site of injection.

Although augmentation can be adequately explained on the basis of delayed absorption, this does not mean that in each instance the mechanism of delaying absorption is the same. Bischoff[17] has demonstrated that zinc sulfate delays absorption by precipitating the gonadotrophic hormones. Merthiolate augments by causing a local reaction at the site of injection[18]. Tannic acid retards absorption by forming an insoluble tannate of the gonadotrophic hormone[19]. Sodium lauryl sulfate produces an edematous reaction, thus forming a depot of hormone containing fluid which is slowly resorbed[14].

Augmenters may be classed as "strong" or as "weak" according to the degree of augmentation they produce. The following materials have been shown to be capable of causing a three to ten-fold increase in gonadotrophic potency and thus may be termed "strong" augmenters: aluminum, zinc, magnesium and ferric hydroxides[6]; zinc sulfate[3]; yeast extract, yeast ash and copper salts[20]; male urine extract, milk, egg white, lemon, horse thyroid and beef liver[7]; whole blood; red blood cells, hemoglobin, hemin and heme[21];

sodium lauryl sulfate(4); tannic acid(19); and merthiolate(18). "Weak" augmentors, which cause a barely detectable to two-fold increase in gonadotrophic potency, include the following substances; casein(22); serum(23); plasma(5) leukocytes(24); and chlorophyll(25).

The assay methods used in demonstrating augmentation have usually involved a three to five day injection period. Enhanced gonadotrophic potency has been noted if the material in question has been mixed with the hormone during the injection period but not if the materials have been kept separated and injected at separate sites. Thus, the material added could be acting as an augmentor, as a protector or as both. It is not probable, considering the degree of increase produced, that protection can fully explain the gonadotrophin enhancing action of strong, "augmentors". It is possible, however, that protection against inactivation accounts for part of the increased gonadotrophic potency. In the case of "weak augmentors" it is possible that protection is the mechanism involved.

Separation of the three gonadotrophin enhancing mechanisms: By definition, synergism is an in vivo mechanism whose locus of action is the gonad, augmentation is an in vivo mechanism whose locus of action is the site of injection, and protection is an in vitro mechanism. This is the basis for identifying the mechanism involved when an increase in potency is noted upon the addition of a given substance to a gonadotrophic hormone solution. Identification of the mechanism involved can be accomplished by the following three procedures:

1. Injection of the substance in question and gonadotrophin at different sites, the materials being kept separate while in vitro.

2. Addition of the substance in question to the gonadotrophin just prior to each injection.

3. Addition of the substance in question to the known gonadotrophin during the entire 3 to 5 day period of injection.

The mechanism involved is identified as follows:

a. If an increase is noted with procedures 1, 2 and 3--the mechanism involved is synergism.

b. If an increase is noted only with procedures 2 and 3, the mechanism involved is augmentation.

c. If an increase is noted only with procedure 3, the mechanism involved is protection against inactivation.

SUMMARY AND CONCLUSIONS

To the two known gonadotrophin enhancing mechanisms, augmentation and synergism, can be added a third--protection against inactivation.

Rat and sheep gonadotrophins are completely inactivated by shaking at 37°C. for 2.5 hours, but only slightly inactivated by incubation at 37°C. for 2.5 hours, or by shaking for 2.5 hours at 3°C.

Addition of whole blood, egg white, testis slices, brain slices or aluminum hydroxide to rat pituitary gonadotrophin offers protection

against inactivation from shaking at 37°C. for 2.5 hours.

Addition of aluminum hydroxide protects sheep pituitary gonadotrophin against inactivation from shaking at 37°C. for 2.5 hours.

Storage at 3°C. for 3 days slightly inactivates rat pituitary gonadotrophins. Addition of whole blood protects against such inactivation.

In vitro inactivation must be considered in concluding that a substance acts as an augmentor, especially since some of the known augmentors such as whole blood, egg white and aluminum hydroxide are also protectors.

BIBLIOGRAPHY

1. Leonard, S. L. Increased stimulation of immature rat ovaries by combined injections of prolan and hypophyseal sex hormone. Proc. Soc. Exper. Biol. and Med., vol. 30, pp. 403-404, 1932.

2. Evans, J. S., Hines, L. R., Geitham, J. J. and Koch, F. C. Comparative study on the augmentation of the action of gonadotropins from various sources in the rat, mouse and chick. Endocrinology, vol. 26, pp. 1012-1021, 1940.

3. Maxwell, L. C. The quantitative and qualitative ovarian response to distributed dosage with gonadotropic extracts. Am. J. Physiol., vol. 110, pp. 458-463, 1934.

4. Bischoff, F. The influence of sodium lauryl sulfate on the biologic response to the gonadotropins and to insulin. Am. J. Physiol., vol. 145, pp. 123-129, 1945.

5. Casida, L. E. Relative gonadotropic augmentative action of plasma and formed elements from blood of cattle. Proc. Soc. Exper. Biol. and Med., vol. 33, pp. 570-572, 1936.

6. McShan, W. H. and Meyer, R. K. Augmentation of sheep pituitary gonadotrophin by insoluble metallic hydroxides. Proc. Soc. Exper. Biol. and Med., vol. 59, pp. 239-242, 1945.

7. Hellbaum, A. A. Augmentation of ovary-stimulating action of gonadotropic preparations. Proc. Soc. Exper. Biol. and Med., vol. 33, pp. 568-570, 1936.

8. Heller, C. G. and Chandler, R. E. Gonadotropic hormone: Modification of the alcohol-precipitation assay method. J. Clin. Endocrinol., vol. 2, pp. 252-253, 1942.

9. Rowlands, I. W. Stability of anterior pituitary extract in aqueous solution. Quart. J. Pharm. and Pharmacol., vol. 8, pp. 642-645, 1935.

10. Van Dyke, H. B. and Wallen-Lawrence, Z. Further observations on the gonad-stimulating principle of the anterior lobe of the pituitary body. J. Pharmacol. and Exper. Therap., vol. 47, pp. 163-181, 1933.

11. Askew, F. A. and Parkes, A. S. On the thermostability of prolon. Biochem. J., vol. 27, pp. 1495-1497, 1933.

12. D'Amour, F. E. and D'Amour, M.C. The potency of certain commercial hormone preparations. Endocrinology, vol. 22, pp. 583-587, 1938.

13. Wu, H. and Ling, S. M. Studies on denaturation of proteins. V. Factors controlling coagulation of proteins by shaking. Chinese J. Physiol., vol. 1, pp. 407-430, 1927.

14. Langmuir, I. and Schaefer, V. J. Properties and structure of protein monolayers. Chem. Rev., vol. 24, pp. 181-202, 1939.

15. Fevold, H. L., Hisaw, F. L. and Leonard, S. L. The gonad stimulating and the lutinizing hormones of the anterior lobe of the hypophesis. Am. J. Physiology, vol. 97, pp. 291-301, 1931.

16. Meyer, R. K. and McShan, W. H. Effect of increasing the number of daily injections of gonadotropic preparations on the ovaries of immature rats. Endocrinology, vol. 29, pp. 31-34, 1941.

17. Bischoff, F. Factors influencing the augmentation effects produced by zinc or copper when mixed with gonadotropic extracts. Am. J. Physiol., vol. 121, pp. 765-770, 1938.

18. Chen, G. and Van Dyke, H. B. The action of merthiolate on the gonadotropic effect of anterior pituitary extract. J. Pharmacol. and Exper. Therap., vol. 62, 333-345, 1938.

19. Bischoff, F. The influence of divided dosage of gonadotropic extracts in the immature male rat. Am. J. Physiol., vol. 114, pp. 483-487, 1936.

20. Fevold, H. L., Hisaw, F. L. and Greep, R. Augmentation of the gonad stimulating action of pituitary extracts by inorganic substances, particularly copper salts. Am. J. Physiol., vol. 117, pp. 68-74, 1936.

21. McShan, W. H. and Meyer, R. K. Heme containing fractions of blood as related to the augmentation of pituitary gonadotrophic extracts. Am. J. Physiol., vol. 119, pp. 574-579, 1937.

22. Saunders, F. J. and Cole, H. H. Means of augmenting the ovarian response to gonadotropic substances. Proc. Soc. Exper. Biol. and Med., vol. 33, pp. 505-508, 1936.

23. Cole, H. H. and Hart, G. H. Concerning gonadotropic substances in mare serum. Proc. Soc. Exper. Biol. and Med., vol. 32, pp. 370-373, 1934.

24. Kraatz, C. P. A possible role of the eosinophil leucocytes in the endocrine complex of the female rat. Am. J. Physiol., vol. 117, pp. 250-256, 1936.

25. Breneman, W. R. Augmentation of pituitary gonadotropic hormones by chlorophyll, plant growth hormones and hemin. Endocrinology vol. 24, pp. 488-493, 1939.