INACTIVATION OF GONADOTROPHINS

IV. THE EFFECT OF PERIODATE IONS AND "BLOOD-GROUP ENZYME" ON THE BIOLOGICAL ACTIVITY OF CHORIONIC GONADOTROPHIN

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[*Manuscript received November* 3, 1952]

Summary

Treatment of chorionic gonadotrophin with periodate ions modified the hormone so that it was no longer inactivated by influenza virus. The dose response curve of the treated hormone was less steep than for untreated, which precluded any accurate comparison. However, at low doses no loss was detected.

Chorionic gonadotrophin was inactivated by a preparation of "blood-group enzyme."

I. INTRODUCTION

In a previous paper (Whitten 1950) it was shown that serum gonadotrophin was rapidly, but not completely, inactivated by dilute solutions of potassium periodate. The residual activity was distinct from that of untreated hormone, since it was no longer inactivated by influenza virus and produced a greater degree of luteinization than comparable doses of the untreated hormone as judged by ovarian weight increase. However, no departure from parallelism between the dose response curves of the untreated and treated hormone was observed, and subdivision of the dose did not increase the response. This was the first indication that the follicle-stimulating and the luteinizing effects of serum gonadotrophin could be dissociated.

The biological activity of serum gonadotrophin is one which produces follicle growth. However, there is some luteinizing activity and the total effect is one which at appropriate doses in hypophysectomized animals closely resembles the normal sequence of events of follicle growth, rupture, and formation of corpora lutea. Chorionic gonadotrophin, on the other hand, does not produce follicle growth in the absence of endogenous pituitary hormones, but results in luteinization of existing follicles and a stimulation of the interstitial cells.

Chemically, these hormones are strikingly similar, as is evidenced by their inactivation by proteolytic enzymes, taka-diastase, and saliva (Abramowitz and Hisaw 1939; Evans and Hauschildt 1942; Whitten 1947; Rimington and Rowlands 1950), by receptor-destroying enzyme and influenza virus (Whitten 1948*a*, 1948*b*), and, as will be shown later, by the "blood-group enzyme" of Morgan (Whitten 1949; Friedmann 1949). Nevertheless, differences have been detected (Li, Simpson, and Evans 1939; Bischoff 1942, 1946) which show that chorionic

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gonadotrophin is more resistant to nitrous acid, ketene, and to heat inactivation.

In view of the interesting results with periodic oxidation of serum gonadotrophin, and since chorionic gonadotrophin similarly contains a large carbohydrate moiety (Gurin, Bachman, and Wilson 1940), the effect of periodate on the latter was studied.

II. Methods and Material

Chorionic gonadotrophin.—This preparation was kindly supplied by Organon, England, and contained 585 I.U. per mg.

Influenza virus.—Allantoic fluid from chick embryos which had been infected with adapted LEE-B was kindly supplied by Dr. A. Isaacs of the World Influenza Centre.

"Blood-group enzyme."—This preparation was kindly supplied by Dr. W. T. J. Morgan of the Lister Institute. The preparation was dissolved in saline and heated at 56°C. for 1 hr.

Periodate solutions.—Potassium periodate of A.R. purity was prepared fresh each day, such that the solution was M/500 in M/15 phosphate buffer pH 6.0.

The remainder of the reagents used have been previously described in this series.

Animals and assays.—The technique of hormone assaying was similar to that described in the British Pharmacopœia (1948). The animals used were hooded rats from the Medical Research Council colony at Mill Hill, and were within the weight range of 40-50 g.

The dose response curve for this colony was established using a standard preparation and four groups of 10 animals at the following dose levels: 3, 9, 27, and 81 I.U. The dose response curve exhibited a linear regression of high significance ($P < 0.001$), without significant departure from linearity and producing mean ovarian weights from 20 to 76 mg. The ratio of the slope of this curve to its standard error was low, and therefore fiducial limits of error were calculated.

Experimental procedure.—Chorionic gonadotrophin was prepared at the beginning of each experiment in distilled water containing a few drops of chloroform, and the solution stored at 2°C. Each day equal volumes of the hormone and periodate solutions were mixed and were allowed to react for 30 min. at room temperature. After this period excess periodate was reduced by the addition of glucose. Control preparations were made by mixing hormone solution with periodate which had previously been reduced by glucose.

III. Observations

Preliminary observations indicated that reduced periodate had no appreciable effect on chorionic gonadotrophin. An assay conducted with untreated hormone against hormone treated with reduced periodate gave percentage recovery 94.07 with fiducial limits ($P = 0.05$) 60.38 and 150.71.

(*a*) *Effect of Periodate on Chorionic Gonadotrophin*

The effect on chorionic gonadotrophin was then studied in two experiments, the results of which are given in Table 1. In each case the dose response curves did not depart significantly from parallelism, and it will be seen that there is no appreciable loss of activity.

TABLE 1

PERCENTAGE OF ACTIVITY OF CHORIONIC GONADOTROPHIN RECOVERED AFTER TREATMENT WITH EXCESS PERIODATE IONS FOR 30 MIN.

Type of Assay	Percentage Recovery	Fiducial Limits of Error $P=0{\cdot}01$
Four-point ..	94·1	59·1-239·0
Six-point ..	86·4	73·3-139·7

Two attempts were then made to improve the accuracy of the assay by increasing the dose interval, but this proved unsuccessful since the dose response curves were no longer parallel ($P < 0.001$). In each case, as shown in Table 2, the lower dose groups were not significantly different, even though significant ovarian growth did occur, whereas the responses to the higher doses of the treated hormone were significantly lower than those of the untreated hormone ($P < 0.001$). In all cases comparable uterine weight increases were observed.

TABLE 2

MEAN OVARIAN WEIGHTS OBTAINED FOR TWO STANDARD FOUR-POINT ASSAYS OF CHORIONIC GONADOTROPHIN TREATED WITH PERIODATE IONS AGAINST UNTREATED HORMONE

Assay	Dose (mg.)	Treatment	Mean Ovarian Wt. (mg.±S.E.)
1	0·045	Treated	19·7±0·95
	0·045	Untreated	20·8±0·80
	0·135	Treated	32·3±1·84
	0·135	Untreated	48·9±2·21
2	0·045	Treated	21·7±0·89
	0·045	Untreated	23·0±1·34
	0·135	Treated	35·2±2·27
	0·135	Untreated	52·4±3·12

(*b*) *Effect of Influenza Virus on the Treated Hormone*

In this experiment comparison of ovarian responses at a single dose level was made between animals injected with oxidized hormone, and similar hormone incubated with influenza virus. The mean ovarian weights for these

groups were respectively 33.3 ± 2.7 mg. and 25.8 ± 1.4 mg., which are not significantly different, whereas both were significantly different ($P < 0.001$) from the response obtained with an equivalent amount of unoxidized hormone incubated with virus (mean ovarian weight 15.7 ± 1.6 mg.). It is probable that some inactivation of endogenous gonadotrophin occurred when the active virus was injected into the test animals, which may account for the slight but not significant decrease of the response to oxidized hormone with virus.

(*c*) *Effect of "Blood-group Enzyme" on Chorionic Gonadotrophin*

Since it has been shown that serum gonadotrophin is rapidly inactivated by the enzyme preparation of Morgan (Whitten 1949; Friedmann 1949), it was decided to examine the effect of this on chorionic gonadotrophin. The enzyme was used as previously described, and again the comparison was made at one dose level between untreated hormone incubated with boiled enzyme and hormone incubated with the active enzyme preparation. The mean ovarian responses obtained were respectively 47.0 ± 5.4 mg. and 11.0 ± 0.5 mg. Analysis of the ovarian responses showed a significant difference ($P < 0.001$); in addition the latter mean ovarian weight was not different from that of untreated animals (11.5 ± 0.4 mg.), which indicates complete inactivation of the hormone.

IV. Discussion

Gaarenstroom and de Jongh (1946) report that it is generally agreed that only one active component is contained in chorionic gonadotrophin. This is supported by the work of Claesson *et al.* (1948), who have prepared electrophoretically homogeneous chorionic gonadotrophin in crystalline form. In view of these findings it is interesting that the biological activity can be modified so that the dose response curve is no longer parallel to that of untreated hormone, while the preparation retains its characteristic gonadotrophic action as shown by ovarian and uterine weight increases. The close agreement of the responses at low doses makes it unlikely that differences in absorption from the injection site, excretion, inactivation, or adsorption by the ovary from the blood could account for the decreased slope. This suggests that the hormone has two or more functions which vary in their susceptibility to periodate. That which is responsible for initial ovarian development is unaltered, while subsequent growth is retarded. However, it must be kept in mind that the response measured in these assays is the result of synergism between chorionic gonadotrophin and endogenous pituitary hormone.

In the many studies on the reactions of chorionic gonadotrophin, only Bischoff (1946) has made any attempt to compare the dose response curves before and after treatment. His observation that the products of heat denaturation were of reduced biological activity, but qualitatively and quantitatively identical with the original hormone, contrasts with the above findings. The detection of such a change in slope, while its significance is not understood, emphasizes the necessity for two or more point assays in these investigations.

In view of the previous findings with serum gonadotrophin in which it was shown that the luteinizing component was virtually unaffected with periodate,

it is interesting that chorionic gonadotrophin, which is considered normally an interstitial cell-stimulating hormone, should not be altered so drastically, and supports the reports referred to earlier that this hormone is the more stable.

That periodic oxidation should again protect a gonadotrophin against destruction by influenza virus suggests that it may be possible to alter the hormone molecule in such a way that biological activity is apparently increased because it is no longer inactivated or excreted in the usual way. Perhaps this is the explanation of the progonadotrophic action of some immune sera.

The similarity between serum and chorionic gonadotrophins is given emphasis by their reactions with the "blood-group enzyme." The significance of this finding must await the characterization of the components of this enzyme preparation.

V. Acknowledgments

I am indebted to Dr. A. S. Parkes, F.R.S., of the National Institute for Medical Research, Mill Hill, in whose laboratory this work was carried out.

VI. References

Abramowitz, A. A., and Hisaw, F. L. (1939).—*Endocrinology* **25**: 633.
Bischoff, F. (1942).—*Endocrinology* **30**: 525.
Bischoff, F. (1946).—*J. Biol. Chem.* **165**: 399.
Claesson, L., Högberg, B., Rosenberg, T., and Westman, A. (1948).—*Acta Endocr.* **1**: 1.
Evans, J. S., and Hauschildt, J. D. (1942).—*J. Biol. Chem.* **145**: 335.
Friedmann, R. (1949).—*Nature* **164**: 626.
Gaarenstroom, J. H., and de Jongh, S. E. (1946).—"A Contribution to the Knowledge of the Influences of Gonadotrophic and Sex Hormones on the Gonads of Rats." "Research in Holland." Vol. 7. (Elsevier Publ. Co. Inc.: New York.)
Gurin, S., Bachman, C., and Wilson, D. W. (1940).—*J. Biol. Chem.* **133**: 467.
Li, C. H., Simpson, M. E., and Evans, H. M. (1939).—*J. Biol. Chem.* **131**: 259.
Rimington, C., and Rowlands, I. W. (1950).—*Nature* **165**: 366.
Whitten, W. K. (1947).—*Aust. J. Sci.* **10**: 83.
Whitten, W. K. (1948*a*).—*Aust. J. Sci. Res.* B **1**: 271.
Whitten, W. K. (1948*b*).—*Aust. J. Sci. Res.* B **1**: 388.
Whitten, W. K. (1949).—*Nature* **163**: 534.
Whitten, W. K. (1950).—*Aust. J. Sci. Res.* B **3**: 346.