THE EFFECT OF CYSTEINE ON GONADOTROPIC HORMONES*

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In the investigation of biologically active substances, major interest attaches to the relation between chemical structure and specific biological activity. One of the best approaches to this problem is given by the study of the effect on biological properties of slight but chemically well defined changes in the molecule. Few methods are available for achieving this in proteins, but fortunately a highly selective effect is caused by the action of cysteine. Most investigators who have studied the reaction of this amino acid with proteins have agreed that it has only one effect: when employed in excess at an alkaline pH it reduces the cystine —S—S— cross-links in the protein (1–3); the thiol (—SH) form of the protein results and a corresponding amount of the cysteine is oxidized to cystine. Papain, urease, and other hydrolytic enzymes have been shown to be activated by cysteine and are, therefore, believed to have reducible —S—S— bonds (4). On the other hand, two highly potent proteins, insulin (2–5) and crotoxin (6), are completely inactivated by this treatment; these are apparently only active when the original —S—S— bridges are intact. The instability of these substances to weak alkali has also been attributed to the lability of —S—S— bonds, in this case

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toward alkaline hydrolysis (7). For three other active proteins with presumably smaller molecular weights, pitocin, pitressin (8), and cobra neurotoxin (9), the presence of S–S– bonds has also been demonstrated; the substances last mentioned, however, when reduced by cysteine, remain biologically active and it is therefore evident that the oxidation-reduction state of the sulfur in proteins may or may not have an effect on their biological activity.

A study of the effect of cysteine on the anterior pituitary and related hormones is indicated, for, through it, marked chemical differences in these hormones may be detected. It has, in fact, already been shown that the growth hormone can be thus freed of several other biologically active contaminants (10), whereas the effect of cysteine on lactogenic hormone will be described shortly elsewhere.

The effect of cysteine on gonadotropic hormones either from the pituitary or from other sources has now been investigated. Cysteine was permitted to act on the hormone solutions at pH 7.7 for 2 days at 23°, the ratio of cysteine to protein being 40:1. To ascertain that the observed effects were due to the reducing action of the cysteine and not to the slightly alkaline pH, control solutions of these preparations were kept at the same pH and temperature for the same length of time. In many cases it proved necessary to add a preservative.

EXPERIMENTAL

Hormone Preparations—The unfractionated gonadotropic preparations were obtained by some modification of methods already published by Jensen et al. (11) and more particularly as follows: 40 per cent alcohol extract of acetone-dried sheep pituitaries was precipitated with alcohol and dried. The powder was extracted with water and the hormones precipitated from this solution by addition of an equal volume of acetone and acetic acid to pH 4.4. From the precipitate all gonadotropic activity was extracted with 1 per cent sodium chloride. The minimal effective dose for follicular stimulation in normal rats for this type of extract is 0.1 to 0.15 mg., for antagonism 0.01 mg., and for repair of the ovarian interstitial tissue in hypophysectomized rats about 0.05 mg. The minimal effective doses for follicular stimulation in
hypophysectomized rats and for 100 per cent augmentation when combined with prolan in normal rats are, for this type of preparation, about the same as for follicular stimulation in normal rats; e.g., 0.1 mg.

Follicle-stimulating hormone preparations which have been used in this investigation were obtained by the following procedure. The unfraccionated extract mentioned above was fractionated with ammonium sulfate; the fraction which precipitates between 0.5 and 0.67 saturation was reprecipitated three times and dialyzed. From this solution follicle-stimulating hormone with increased potency can be obtained by precipitation at pH 4.1 with an equal volume of acetone. After the precipitate is redissolved in water, it can be further purified by bringing the aqueous solution again to pH 4.1 and discarding the precipitate. By repeating this procedure, follicle-stimulating fractions can be regularly obtained which show a minimal effective dose for follicular stimulation of 0.02 to 0.025 mg. in normal and 0.008 mg. in hypophysectomized rats; this same level gives 100 per cent augmentation when combined with prolan in normal rats; the minimal effective dose for antagonism in normal rats is 0.015 mg.; interstitial tissue repair in hypophysectomized rats becomes evident only when about 20 to 50 units are injected intraperitoneally.

Interstitial cell-stimulating hormone preparations were kindly supplied by Dr. C. H. Li of this department. The minimal effective dose for antagonism and for repair of the interstitial tissue of the hypophysectomized rat was 0.01 mg.

Cysteine Treatment—All preparations were used in aqueous solution in phosphate buffer at pH 7.7. As controls, samples were kept in this solvent for 2 days at room temperature without addition of cysteine. It was observed that some of the preparations showed varying degrees of inactivation under these conditions (follicle-stimulating hormone from pituitary, all preparations from pregnancy urine, and gonadogen), while others did not (unfraccionated pituitary preparations, interstitial cell-stimulating hormone, and gonadin). This inactivation could be com-

\[1\] It may be remarked in passing that the inactivation of follicle-stimulating hormone from pituitary by cysteine proceeds equally well when 40 per cent urea buffered to pH 7.7 is used as a solvent. No inactivation is caused by the urea solution alone.
pletely prevented by the addition of preservatives to the solutions (thymol, 2 per cent butanol). Cysteine neutralized with sodium carbonate was employed at 40 times the amount of protein. The solutions were kept in tightly stoppered flasks with little air space; autoxidation to cystine was negligible under these conditions and no special precautions for the exclusion of oxygen had to be taken. After 2 days treatment, the solutions were, in a few cases, dialyzed to remove the cysteine but no difference was found when non-dialyzed solutions were injected. Preparations which were inactivated by cysteine were similarly inactivated in the presence of preservatives. Preparations which were not inactivated by the chemical action of cysteine did not deteriorate through bacterial growth and hence it was unnecessary to add a preservative to these solutions. A curious and unexplained observation was made in this connection: thymol was found to protect antuitrin-S and follutein against bacterial growth; cysteine had the same effect; when both were combined, however, inactivation occurred, so that it seems likely that thymol reacted with the cysteine. When 2 per cent butanol was used instead of thymol, no inactivation of these preparations was observed with or without cysteine.

**Biological Standardizations**—Methods were essentially the same as have been previously reported (12). In tests for follicle-stimulating hormone the solutions were injected subcutaneously; for interstitial cell stimulation or antagonism, the injections were intraperitoneal. The dose which gave follicular and uterine development in two out of three normal, immature rats was taken as the minimal effective dose for follicle-stimulating hormone. A one-fourth reduction in the ovarian weights, which was produced by a standard amount of pregnant mare serum, was regarded as the minimal effective dose for antagonism when the solution to be tested for antagonizing power was injected simultaneously with the pregnant mare serum. The principle from pregnancy urine was injected subcutaneously; that from pregnant mare serum intraperitoneally.

**Results**

As Tables I and II show, all pituitary gonadotropic substances, unfractionated, follicle-stimulating, and interstitial cell-stimu-
lating preparations were so completely inactivated that the minimal effective dose of the follicle-stimulating hormone, for instance, was now 40 times and for interstitial cell-stimulating hormone over 100 times its original level. Gonadotropic fractions from normal male and female menopause urine (prospermin and gamone) were reduced to less than 10 per cent of their original potency by this treatment. On the other hand, cysteine did not inactivate gonadotropic preparations from pregnant mare serum (gonad, gonadogen) or from human pregnancy urine (antuitrin-S, follutein2).

These results show that a striking difference exists between the hormones isolated from the pituitary or from the urine of normal

2 Follutein showed a slight decrease in potency on cysteine treatment.

**Table I**

*Inactivation of Pituitary Gonadotropic Hormones by Cysteine*

The figures represent the minimal effective dose, in mg.; f. s. h. and i. c. s. h. refer to the follicle-stimulating and the interstitial cell-stimulating hormones respectively.

<table>
<thead>
<tr>
<th>Gonadotropic hormone preparation</th>
<th>Before cysteine treatment*</th>
<th>After cysteine treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal rats</td>
<td>Hypophysectomized rats</td>
</tr>
<tr>
<td></td>
<td>F. s. h.</td>
<td>Antagonism</td>
</tr>
<tr>
<td>Unfractionated</td>
<td>0.15</td>
<td>(0.1)</td>
</tr>
<tr>
<td>Follicle-stimulating</td>
<td>0.075†</td>
<td>(0.075)</td>
</tr>
<tr>
<td>Interstitial cell-stimulating</td>
<td>0.2</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* All values except those in parentheses are for “control solutions,” kept for the same length of time at the same pH and temperature as those treated with cysteine.
† To these solutions 2 per cent butanol was added, which prevented loss of activity in the controls.
men and women, on the one hand, and the hormones found in pregnancy whether in the urine of women or blood stream of mares, on the other hand. The conclusion must be drawn that disulfide bonds are present in the gonadotropic hormones of pituitary origin and that these bonds are essential for the activity of the hormones, while the gonadotropic hormones of placental origin either

### Table II

**Resistance of Gonadotropic Hormones of Pregnancy Urine and Pregnant Mare Serum against Cysteine**

The figures represent the minimal effective dose, in mg.

<table>
<thead>
<tr>
<th>Pregnancy urine</th>
<th>Control, pH 7.7, 2 days, 23°</th>
<th>Cysteine-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>For follicle-stimulating hormone</td>
<td>For interstitial tissue repair*</td>
</tr>
<tr>
<td>Antuitrin-S</td>
<td>0.008 (Thymol) (0.008)</td>
<td>0.010 (&lt; &lt;0.025 (Butanol)</td>
</tr>
<tr>
<td>Follutein</td>
<td>&lt; &lt;0.025 (Butanol)</td>
<td></td>
</tr>
<tr>
<td>Prolan†</td>
<td>0.075 (0.015)</td>
<td>0.05 (Butanol)</td>
</tr>
<tr>
<td>Prolan†</td>
<td>0.025 (Thymol) 0.015 (Butanol)</td>
<td>0.05</td>
</tr>
<tr>
<td>Prolan†</td>
<td>&gt;0.25 (0.05)</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

The values in parentheses are for fresh solutions, not kept for 2 days at pH 7.7. When preservatives were used, these are indicated.

* Hypophysectomized rats.
† Prepared by Dr. H. Jensen in this department.

contain no such bonds or are equally potent in the thiol form. The inactivation of prospermin and gamone by cysteine places these preparations in the "pituitary" group of gonadotropic substances

3 The former is the more likely assumption in view of K. Meyer's statements that prolan is free from sulfur and pregnant mare serum free from cysteine (13).
and it is indeed difficult to conceive a source of origin for them other than the pituitary. The biological properties of pregnant mare serum resemble both those of pregnancy urine and of pituitary gonadotropic hormones; the resistance of the principle from pregnant mare serum to cysteine, however, would indicate that it, like the principle from pregnancy urine, is secreted by the placenta.

The marked difference in the effect of cysteine on placental and pituitary gonadotropic principles seems to us evidence of a fundamental difference in the chemical structure of these two groups of hormones.

SUMMARY

It has been possible to demonstrate a marked difference in the effect of cysteine on hypophyseal as contrasted with placental, gonadotropic hormones. Hypophyseal gonadotropic hormones as well as those found in the urine of normal men and women are completely inactivated by this reagent, whereas neither the principle from human pregnancy urine nor that found in the blood stream of pregnant mares shows loss of potency under the same conditions.

BIBLIOGRAPHY

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