Inactivation of follicle-stimulating hormone by enzymic release of sialic acid

In 1948 it was observed by WHITTEN[1] that the biological activity of crude chorionic and pituitary gonadotropins was lost when the hormone materials, prior to injection into animals, were treated with influenza virus or with the receptor-destroying enzyme (RDE) of *Vibrio cholerae*. Over the last years the influenza-virus enzyme and RDE have been recognized[2] as neuraminidases and their action defined[3] as "cleaving the α-ketosidic linkage joining the terminal acylated neuraminic acid to another sugar or sugar derivative". Recently BROSSMER AND WOLTER[4] confirmed WHITTEN's observation and reported that the loss of biological activity of chorionic gonadotropin on treatment with the above catalytic agents was accompanied by the release of N-acetylneuraminic acid, as shown by paper chromatography. Since their work was carried out on a qualitative basis only and since the influenza virus and RDE used were crude preparations, it seemed desirable to investigate quantitatively the effect of highly purified α-neuraminidase on the chemical composition and biological activity of a standard preparation of FSH.

25 mg of the ovine FSH preparation* was dissolved in 4.0 ml water. 3 ml of the solution were treated with highly purified RDE** (50,000 units, 35 μg protein) at pH 6.0 and in the presence of 0.005 *M* Ca^{++} at 35° for 18 h. The control (residual 1.0 ml of solution) was kept under the same conditions but without RDE. Control and assay were then quantitatively transferred to dialysing tubings and dialysed against 10 vol. water at 0° for 48 h. The dialysates were lyophilized. Sialic acid determinations[5] on aliquots of the contents of the dialysing bags and on aliquots of the lyophilized materials gave the results shown in Table I.

TABLE I

RELEASE OF SIALIC ACID BY NEURAMINIDASE FROM FSH

	Dialysed material		*Dialysate*	
	FSH	*FSH-RDE*	*FSH*	*FSH-RDE*
Sialic acid content*	5.0	0	0	5.15

* mg sialic acid (expressed as N-acetylneuraminic acid) obtained/100 mg FSH.

The enzymically released compound was identified as sialic acid by conversion to pyrrole-2-carboxylic acid (0.1 *N* NaOH, 100°, 20 min) using the isolation procedure described previously[6]. The isolated compound (solvent, ethanol) had a u.v. absorption maximum at 263 mμ identical with that of authentic pyrrole-2-carboxylic acid. The identity of the two compounds was confirmed by paper chromatography (*n*-butanol–pyridine–water (6 : 4 : 3, v/v)).

The gonadotropic activity of the control and assay (RDE-treated, dialysed FSH) was examined in 3-week-old female mice. A group of 6 animals was injected with one

Abbreviations: FSH, follicle-stimulating hormone; RDE, receptor-destroying enzyme.

* The FSH preparation, code name NIH-FSH-S-1, was a gift from the National Institutes of Health, Bethesda, U.S.A.

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of five graded doses of either control (0.001–0.1 mg FSH) or assay (0.01–0.9 mg); the mice also received 40 I.U. of chorionic gonadotropin[7]. After appropriate time the ovarian and uterine weights were recorded. Typical dose-response regressions were obtained with 0.03 mg and more FSH, whereas the highest dose (0.9 mg) of the RDE-treated FSH used did not cause any significant increase in ovarian and uterine weights. Similar results were obtained with rats (without simultaneous application of chorionic gonadotropin).

The following conclusions may be drawn from the experimental results. (a) All sialic acid residues in FSH are terminal, linked α-ketosidically to their partner and are accessible to α-neuraminidase. (b) Enzymic release of the sialic acid residues reduces the biological activity of FSH by 97 % or more. A more exact estimate of the residual biological activity, if any, would have required amounts of pure FSH not available at present. The data presented taken together with the well known capacity of certain mucoproteins to inhibit in high dilution haemagglutination by influenza viruses, a capacity lost on treatment with α-neuraminidase, are some indication of the range of biological activities imparted to mucoproteins by the presence in them of terminal sialic acid residues.

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Metabolism of [4-^{14}C]corticosterone by fibroblasts, strain U12-79

The physiological importance of the fibroblast and its ability to resist destruction in the presence of corticosteroids in an inflammatory process has been shown by DOUGHERTY *et al.*[1,2]. It has been demonstrated that the ability of corticosteroids to inhibit inflammation is a function of structure and that compounds which have a 17α-OH group are the most potent anti-inflammatory steroids[3]. The metabolism of various steroids by connective tissue[4] and fibroblasts propagated *in vitro*[5,6] have been studied in these laboratories. The data to be presented in this report indicate that a permanent strain of fibroblasts converts corticosterone to 11β,20β,21-trihydroxypreg-4-en-3one.

Strain U12–79[7,8] was propagated in solution S103[9] fortified with 5 % dialysed