STIMULATION OF SYNTHESIS OF GLYCOPEPTIDE ENZYMES IN THE RAT UTERUS BY OESTRADIOL*

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SUMMARY

Glycoproteins from immature and immature, oestrogen-stimulated and adult rat uteri were isolated and analysed by chemical and gel electrophoretic methods. Esterase, acid phosphatase, alkaline phosphatase and peroxidase activities were found. Changes in electrophoretic mobilities of certain enzyme bands in polyacrylamide gel were also observed after hydrolysis of the preparations with neuraminidase. These latter observations and chemical analyses provide additional evidence of the carbohydrate nature of the enzymes. The influence of 17β-oestradiol on immature rat uteri caused a significant increase in total protein and sialic acid per uterus compared with controls. Oestrogen treatment also resulted in an increase in the total activity of esterase and acid and alkaline phosphatases per uterus, but there was no increase in specific activities. Observations of electrophoretic patterns of glycoprotein preparations from untreated and oestrogen-stimulated, immature uteri did not show the evolution to a more adult pattern by oestrogen stimulation. These studies show that stimulation with oestrogen increases the synthesis of glycoprotein in the immature rat uterus. Factors which are involved in the more intricate control of glycoprotein biosynthesis need to be elucidated.

INTRODUCTION

Biochemical and histological studies of the uterus have long been used to observe the control of protein metabolism by oestrogens. Experiments with this convenient model cover a wide spectrum, from measurements of protein synthesis after oestrogen stimulation (Telfer, 1953) to the initial changes in RNA metabolism (Telfer, 1953; Ui & Mueller, 1963; Wilson, 1963).

In the course of previous studies on the chemistry of glycoproteins from connective tissues (Dugan, Radhakrishnamurthy & Berenson, 1967), a number of enzyme systems were found in preparations from different sources. These studies suggested that certain glycoproteins might play a significant enzymic role in regulating the meta-
bolism of connective tissue. Other studies (Berenson, Radhakrishnamurthy, Fishkin, Dessauer & Arquembourg, 1966) stressed the fundamental importance of the presence of a family of glycoproteins which were demonstrated to be immunologically reactive and probably genetically determined. Yet, little is known about the factors which influence the amounts and types of glycoproteins found in tissues. For this reason, it was considered interesting to explore the qualitative and quantitative changes in enzymes of a glycoprotein nature from the immature rat uterus under oestrogen stimulation. Esterase and acid and alkaline phosphatases were studied and, in addition, adult uterine glycoproteins were hydrolysed by neuraminidase to characterize the glycoproteins further.

**METHODS**

**Oestrogen stimulation.** Immature female Houston-Cheek (Houston, Texas) rats aged 21 days and adult, multiparous rats aged approximately 180 days were used. All rats were injected with a mixture of 1000 units penicillin and 12·5 mg. streptomycin shortly after arrival at the laboratory. The oestrogen-treated rats (64) were given 50 µg. 17β-oestradiol in 0·1 ml. sesame oil, i.m.; control, immature animals (98) and adults (10) received 0·1 ml. of sesame oil only. Of the oestrogen-treated rats, half were killed 48 hr. and the remainder 72 hr. after injection of the hormone. These groups were studied in triplicate.

**Isolation of glycoprotein material.** The uteri were dissected free of all extraneous tissue and weighed immediately. They were then pooled and extracted for glycoproteins by methods described earlier (Radhakrishnamurthy, Fishkin, Hubbell & Berenson, 1964). Briefly, these consisted of extraction of the tissue with 0·9 % NaCl solution, dialysis of the extract with distilled water, adjustment to pH 4·0, and fractional precipitation with (NH₄)₂SO₄ at 40, 60 and 100 % saturations. The fraction precipitated between 60 and 100 % saturations contained the glycoprotein material. This precipitate was dissolved in water, dialysed against distilled water, and concentrated. Based on biuret analysis, the glycoprotein fraction represented 0·1–0·2 % of the wet weight of the uteri.

**Zone electrophoresis.** Vertical polyacrylamide gel electrophoresis of 20–50 µl. samples containing 300–450 µg. glycoprotein material was carried out in borate buffer, pH 8·6, by methods described previously (Radhakrishnamurthy, Chapman & Berenson, 1963; Berenson et al., 1966). After electrophoresis the gels were stained with amidoblack for proteins and with stains for specific enzymic activities (Dugan et al., 1967). Esterases were localized on gels by incubation in a solution of α-naphthylacetate in tris-maleate buffer, pH 5·0, containing Fast Red TRN (Dajac Laboratories, The Borden Chemical Co., Philadelphia, Penn.) at 38° for 30 min. Acid and alkaline phosphatases were similarly stained with Fast Red TRN after incubation with α-naphthylphosphate in tris-maleate buffer, pH 5·2 for acid phosphatase and 8·6 for alkaline phosphatase. MnCl₂ was used as an activator for phosphatases. Peroxidase was detected on the gel after the gel had been incubated with a dilute solution of H₂O₂ (0·2 % in 0·5 % acetic acid) and stained with benzidine HCl. β-Glucuronidase activity was tested in these glycoprotein preparations with 8-hydroxyquinoline as a substrate. After electrophoresis of the glycoprotein the gels were incubated with the substrate solution in tris-maleate buffer, pH 5·2, containing Fast Red TRN. This
procedure was found satisfactory in detecting \( \beta \)-glucuronidase in glycoprotein preparations from several connective tissues (Dugan et al. 1967); however, no \( \beta \)-glucuronidase was detected in the glycoprotein preparations of rat uteri by this method.

**Analyses.** The glycoprotein preparations were analysed for polypeptides by a biuret method (Mehl, 1945) and for sialic acid by the diphenylamine reaction (Anderson & MacLagan, 1955). Phosphatases were determined by the Sigma Chemical Company's procedure (Technical Bulletin No. 104), and esterase activity was measured by a modification (Dugan et al. 1967) of the method of Kramer & Gamson (1955, 1960) with 2,6-dichloroindophenyl acetate as a substrate.

**Hydrolysis with neuraminidase.** Hydrolysis of the glycoproteins by neuraminidase was carried out in tris buffer, pH 7-7, 0·1 M. To 0·1 ml. glycoprotein solution (45 mg./ml.) were added 0·3 ml. of tris buffer and 0·1 ml. of a solution of neuraminidase (50 units) (Mann Research Laboratories, Inc., New York); the reaction mixture was incubated at 38° for 24 hr. Electrophoretic studies were performed on glycoprotein material before and after hydrolysis.

**Table 1. Adult rat uterus: recovery of enzymes from glycoprotein preparations**

<table>
<thead>
<tr>
<th></th>
<th>Saline extract</th>
<th>Glycoprotein preparation</th>
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<tbody>
<tr>
<td>Total protein (mg.)</td>
<td>630</td>
<td>25·5</td>
</tr>
<tr>
<td>Esterase (units)</td>
<td>245</td>
<td>142</td>
</tr>
<tr>
<td>Acid phosphatase (units)</td>
<td>815</td>
<td>617</td>
</tr>
<tr>
<td>Alkaline phosphatase (units)</td>
<td>890</td>
<td>366</td>
</tr>
</tbody>
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* Thirteen rats were used; the combined weight of the uteri was approximately 10 g.

**RESULTS**

In preliminary experiments, the activities of esterase and acid and alkaline phosphatases were determined in the original extract and in the final preparation of glycoproteins from adult rat uteri (Table 1). The specific activities of all the enzymes in the preparation showed a 12–20-fold increase over the initial values. About 75% of the total acid phosphatase activity, 60% of the esterase activity, and 40% of the alkaline phosphatase activity were recovered. These results indicated that these enzymes were not merely associated with glycoproteins as contaminants, but were glycoproteins (sialoproteins, Table 2).

Quantitative analyses of the glycoproteins from uteri of the four groups of animals are shown in Table 2. The expected increase in uterine weight with oestrogen stimulation is apparent. So is the increase in polypeptide material in the glycoprotein preparations and an increase in sialic acid. The total enzymic activity of the esterases and phosphatases per uterus also increased, although the specific activity (units/mg. protein) showed no consistent pattern of change. The latter characteristic may represent a more subtle change, and would be worthy of further investigation in view of studies showing activation and induction of protein synthesis (Attardi, Naono, Rouviere, Jacob & Gros, 1963).

Gel electrophoretic analysis of the glycoproteins from immature rat uteri before and after stimulation with oestrogen showed no significant differences when the gels were stained either for protein, esterase or phosphatases. As an example, the electro-
phoretic patterns of esterase are shown in the Plate, fig. 1. Except for a small amount of denatured protein at the origin of the 72 hr. sample, no differences are apparent between experimental and control samples. However, the slowest migrating esterase band of adult rat uteri appears to have a different mobility from that of the corresponding esterase of immature rats. Similarly, certain differences between the adult and immature rats were observed in the bands stained for protein. The two leading major protein bands of the material from adult uteri moved faster than the corresponding bands of that from immature uteri. Only one each of acid and alkaline phosphatase bands was detected in each group, and no difference in electrophoretic mobility of the bands was observed. Since the activities of these enzymes were measured by a more accurate colorimetric method, no quantitative staining of the gels was attempted. Although peroxidase activity was not determined quantitatively in these preparations, histochemical staining of the gel after electrophoresis of the glycoproteins showed the presence of this enzyme in all the samples. Only one band was observed in samples from immature rat uteri, but the adult rat uteri showed two bands.

The results of the electrophoresis of the adult uterine glycoprotein material before and after hydrolysis with neuraminidase showed certain changes in the electrophoretic patterns. As an example, a gel stained for esterases is shown in the Plate, fig. 2. Differences in the electrophoretic mobility of the fast-migrating major esterase bands are apparent from the figure. Similarly, changes were observed in electrophoretic mobility in the slow-migrating bands of the gel stained for protein after hydrolysis of the glycoprotein with neuraminidase. Gels stained for alkaline phosphatase and peroxidase showed an additional band with slower mobility than that of the original band in both gels. The results suggest incomplete hydrolysis of these glycoproteins, with the additional bands representing sialic acid-free enzymes. No change was detected in the acid phosphatase bands. However, there was a poor correlation, histochemically, between protein bands and enzyme bands due to differences in sensitivity of the two methods. Hydrolysis by neuraminidase is contributory evidence to the chemical analyses and observations reported earlier (Dugan et al. 1967)
Oestrogen stimulation of glycoprotein synthesis

that many of the proteins studied contain sialic acid. In similar studies alkaline phosphatases from human placenta were recently (Ghosh & Fishman, 1967) characterized as sialoproteins.

DISCUSSION

The results presented demonstrate the acceleration of glycoprotein synthesis in the immature rat uterus by the administration of oestradiol. Whether this increase is part of the rise in protein synthesis generally and in uterine growth is not clear. As a result of this augmented rise in protein synthesis there is an increase in the total glycoprotein enzymic activity in the uterus. A comparison with previous quantitative data on the response of esterase (Harris & Cohen, 1951), phosphatase (Harris & Cohen, 1951; Leathem, 1959) and peroxidase (Lucas, Neufeld, Utterback, Martin & Stotz, 1955) to oestrogens is difficult, since earlier investigators studied all uterine proteins, whereas this investigation has been limited predominantly to a fraction technically enriched with sialic acid.

Histological studies of the effects of oestrogen on uterine enzymes have established that the relative distribution of the enzymes is changed (Atkinson & Elftman, 1947; Watanabe & Fishman, 1964). The observations suggest the possibility of different isoenzymes throughout the tissues with varying sensitivities to the hormonal stimulus. However, this study shows that isoenzymes are increased only in proportion to each other and that no new isoenzymes are formed. The transition towards the adult glycoprotein pattern which was found to be somewhat different from the immature pattern was not detected.

Finally, the neuraminidase experiments with adult tissues show conclusively that some of the commonly investigated enzymes of uterine tissue are glycoproteins.

REFERENCES


**DESCRIPTION OF PLATE**

Fig. 1. Electrophoretic patterns of esterases from immature rat uteri after stimulation with oestrogen. Electrophoresis was carried out in polyacrylamide gel using borate buffer, pH 8.6. A specific stain for esterase was used (for details see text p. 262). There were no marked differences between samples from control and experimental animals. The material remaining at the origin in the 72 hr. sample might be a denatured protein. The marker was bovine serum albumin.

Fig. 2. Electrophoresis of esterases from adult rat uteri after the digestion of the glycoprotein with neuraminidase (NAN-ase). Electrophoretic conditions were similar to those described for fig. 1. Glycoproteins were hydrolysed with NAN-ase in tris buffer, pH 7.7, for 24 hr. at 38° together with the samples used for control and NAN-ase. Original glycoprotein material without incubation was also used in this electrophoretic run for comparison. Note changes in mobilities of esterase after hydrolysis with NAN-ase.