Synthesis of Estrogen-Specific Proteins in the Uterus of the Immature Rat*

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ABSTRACT: Synthesis of estrogen-specific uterine proteins can be detected after treatment of immature rats with 17β-estradiol. Electrophoresis of a mixture of ^{14}C-labeled proteins from hormone-stimulated uteri and ^{3}H-labeled proteins from control uteri allows the detection of hormone-specific proteins by the increased ^{14}C:^{3}H ratio within areas of the gel. The stimulation of hormone-specific synthesis becomes detectable 30 min after 17β-estradiol administration and reaching maximum at 1 hr rapidly declines to control levels 4 hr later. There is no detectable increase in the rate of synthesis of other uterine proteins until after the synthesis of estrogen-specific proteins has begun. Actinomycin D treatment completely prevents the hormone-induced synthesis of estrogen-specific proteins but apparently enhances the synthesis of other proteins of different electrophoretic mobility. The synthesis of estrogen-specific proteins is also observed after *in vitro* treatment of isolated uteri with 17β-estradiol. The estrogen-specific proteins were purified by preparative polyacrylamide gel electrophoresis; electrofocusing studies of this material show them to be a group of acidic proteins with isoelectric points in the range of 3.5 to 4.0.

Some of the biochemical events which occur in the uterus of the immature rat after treatment with 17β-estradiol have been summarized recently (Hamilton, 1968; McKerns, 1967; Segal and Scher, 1967). Most of these effects, such as the increased synthesis of uterine proteins, RNA, phospholipids, etc. (McKerns, 1967; Segal and Scher, 1967), which contribute to the accumulation of these substances in the growing uterus can be considered as secondary effects of the hormone. It is assumed that the primary effect is exerted at some key point(s) of intermediary metabolism, perhaps through the enhanced synthesis of certain enzymes. Notides and Gorski (1966) showed that the synthesis of a class of uterine proteins was markedly enhanced by treatment with 17β-estradiol. We have made similar observations using double-isotope-labeling methods which allow a more quantitative evaluation of the data and more direct interpretation of the possible function of these proteins.

Experimental Procedures

Treatment of Animals. Immature female rats of the St. Louis University colony (20–23-days old, weighing 40–50 g) were injected intraperitoneally with a single dose of 5 μg of 17β-estradiol in aqueous 9.5% ethanol. Control animals were injected with the solvent. Labeled amino acids (Schwarz BioResearch) and actinomycin D (a gift of Merck and Company) were administered in isotonic saline by intraperitoneal injection. Animals were sacrificed by decapitation and the uteri were quickly removed and stripped of fat.

Incubation Conditions. The excised, intact uteri from 7 to 10 animals were placed into 50-ml beakers containing 3 ml of ice-cold Eagle's HeLa tissue culture medium (Difco) which was supplemented with glutamine but not with serum, since the latter was observed to introduce artifact bands in the electrophoretic patterns and did not appear to be essential during the limited incubation periods used in these experiments. L-Leucine-4,5-*t* (final specific activity 400 mCi/mmole) or leucine-^{14}C (final specific activity 28 mCi/mmole) in 0.01 M NaCl was added to the medium, and the tissues were incubated at 37° with shaking under an atmosphere of 95% O_2–5% CO_2 for 1 hr. Under these conditions the incorporation of leucine-*t* into uterine acid-insoluble materials was linear for incubation periods up to 10 hr. After 1-hr incubation the tissues were washed with ice-cold saline and stored at −80° or analyzed immediately. The tissues were homogenized at 4° in 0.15 M KCl (0.05 ml/uterus) with a ground-glass conical tissue grinder (Duall) and centrifuged at 105,000*g* for 60 min. The supernatant fraction was used for electrophoresis.

Electrophoresis. Polyacrylamide gel electrophoresis was performed as described by Ornstein and Davis (1964). Separations were performed at 3.5 mA/tube with a Canalco Model 12 disc electrophoresis apparatus using gels of 6- or 12-cm length and 0.5-cm diameter. The gels were stained with Amido Black and destained electrophoretically.

Preparative gel electrophoresis was performed with the Büchler "Poly-Prep" apparatus. A 7.5% gel of 3-cm height was used for the separating gel. The stacking method described by Hjerten *et al.* (1965) was used in place of the stacking and sample gels.

Measurement of Radioactivity. After recording the positions of the bands, the acrylamide gels were frozen on Dry Ice and cut into slices approximately 1 mm thick with a device consisting of parallel razor blades mounted on threaded rods and separated by washers. Each slice was placed into a scintillation vial and 10 ml of a 1:10 mixture of NCS reagent

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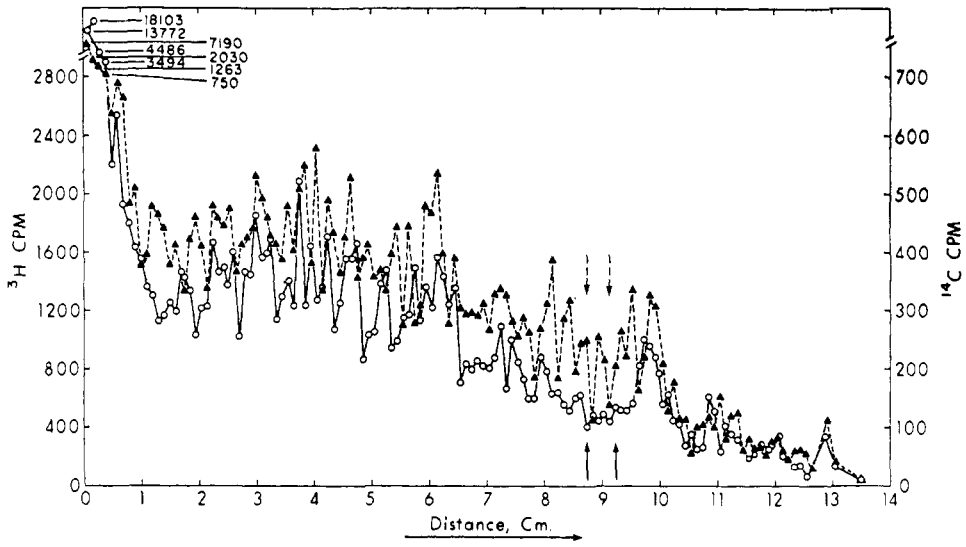

FIGURE 1: Pattern of radioactivity of labeled soluble proteins of control and estrogen-treated uteri electrophoresed on separate gels. Uterine proteins were labeled by incubating the tissues for 1 hr in HeLa medium containing labeled leucine. The solid line represents control uteri which were labeled with leucine-*t*; the dotted line, uteri from animals stimulated with 5 μg of 17β-estradiol 1 hr before sacrifice, followed by labeling with leucine-^{14}C. Aliquots (50 μl) of the 105,000*g* supernatant fraction from each group were electrophoresed simultaneously on separate gels; the gels were sliced and counted. The region between the arrows indicates the position of band 5: ▲—▲, ^{14}C (stimulated); O—O, ^{3}H (control).

(Nuclear-Chicago Corp.) and toluene scintillation fluid (5 g of 2,5-diphenyloxazole + 300 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene in 1 l. of toluene) were added. The vials were capped and allowed to stand overnight at room temperature. During this period the radioactive proteins were extracted from the gel slice. This method allowed quantitative recovery of radioactive proteins from acrylamide gels (Table I).

The radioactivity in fractions recovered from the preparative electrophoresis apparatus was determined by precipitating aliquots of each fraction with 10% trichloroacetic acid, collecting the precipitates on Millipore filters (type HA, 0.45 μ) by suction filtration, and washing the filter five times with 5% trichloroacetic acid. The dried filters were placed in toluene scintillation fluid and counted. All radioactivity determinations were made on a Packard Tri-Carb liquid scintillation spectrometer.

Measurement of the Rate of Synthesis of Estrogen-Specific Proteins. Uteri removed from animals at intervals after injection of the 17β-estradiol were incubated in the leucine-^{14}C medium while control uteri were incubated in the presence of leucine-*t*. Equal numbers of uteri were used for each group in an experiment. Each group of tissues was homogenized and centrifuged separately. An aliquot (usually 25 μl, representing the soluble protein equivalent of 0.5 uterus) of the control ^{3}H group was mixed with an equal volume of each of the stimulated ^{14}C groups and the mixtures were electrophoresed. The gels were sliced and the ^{14}C:^{3}H ratio in each slice determined.

Results

Electrophoresis of Uterine-Soluble Proteins. Polyacrylamide gel disc electrophoresis separates soluble uterine proteins into a large number of sharp bands. The staining intensity of the band referred to as band 5 increases rapidly after estrogen treatment and since its electrophoretic mobility approximates that of rat serum albumin it may represent an increased uptake of albumin into the uterine tissue (Kalman, 1955). Band 5 seems to correspond to zone B in the

TABLE I: Determination of Radioactive Proteins in Polyacrylamide Gels.[a]

Isotope	Dpm Applied ($\times 10^{-3}$)	Dpm Recovered ($\times 10^{-3}$)	% Recovery
^{3}H	432	411	95
^{14}C	50.9	52.2	103
^{14}C	55.7	56.5	101

[a] Uterine-soluble proteins labeled with leucine-*t* or -^{14}C *in vitro* were polymerized into 7% acrylamide gels, stained with Amido Black, electrophoretically destained, sliced, and the radioactivity was measured. The values so obtained were compared with the radioactivity of the same protein solutions determined by precipitating aliquots, containing 2 mg of albumin as carrier, with 5% trichloroacetic acid and washing the precipitate five times with 5% trichloroacetic acid by repeated resuspensions and centrifugations. The precipitates were solubilized in NCS reagent, transferred to scintillation vials, and counted. Efficiencies of counting were determined by internal standardization using toluene-*t* or toluene-^{14}C and by automatic external standardization. Efficiency for ^{3}H was 30% and for ^{14}C, 80% in the samples containing gel slices. The disintegrations per minute recovered refers to the sum of the radioactivity present in the gel slices after efficiency of counting corrections. These recoveries were not altered by electrophoresis of the samples.

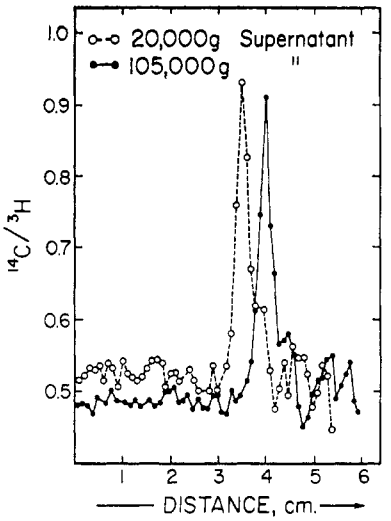

FIGURE 2: Coelectrophoresis of differentially labeled, soluble uterine proteins from control and estradiol-treated animals. Twenty animals were divided into two groups; one group was injected with solvent (controls) the other with a single injection of 5 μg of estradiol 1 hr prior to sacrifice. The uteri from each group were incubated for 1 hr in HeLa medium containing leucine-*t* (controls) or leucine-^{14}C (stimulated). Aliquots (25 μl) of the 20,000*g* supernatant of each group were mixed and electrophoresed. The 105,000*g* supernatants were treated likewise. The gels were sliced and counted, and the ratio of ^{14}C to ^{3}H in each slice was determined.

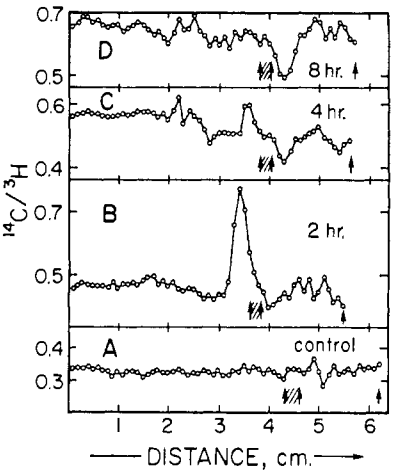

FIGURE 4: Relationship of synthesis of estrogen-specific proteins to time after estradiol treatment. (a) One group of uteri from unstimulated animals was labeled for 1 hr in medium containing leucine-*t;* another identical group was labeled with leucine-^{14}C. Aliquots of the two soluble fractions were mixed and electrophoresed. (b,c,d). The ^{14}C:^{3}H ratios of estrogen-specific proteins were measured at 2, 4, and 8 hr, respectively, after treatment of three groups of animals with a single injection of 5 μg of estradiol. Each group of stimulated tissues was labeled at the end of the prescribed period of hormone treatment by incubation for 1 hr in leucine-^{14}C medium. A single group of controls was labeled for 1 hr in leucine-*t* medium. An aliquot of each of the ^{14}C-labeled soluble proteins was mixed with an equal volume of the ^{3}H-labeled soluble proteins before electrophoresis.

starch gel system reported by Notides and Gorski (1966). The fastest migrating component (band 1) follows only 1–2 mm behind the bromophenol blue tracking dye. Bands 1 and 5 were used as reference bands and their positions are designated by arrows in the electrophoretic labeling patterns described below.

The pattern of radioactivity (Figure 1) of proteins from experimental and control uteri which were labeled for 1 hr *in vitro* and electrophoresed on different gels shows a large number of radioactive peaks and although indications of estrogen-mediated differential labeling are seen in the region between 6.5 and 9.5 cm, the exact nature of this labeling is uncertain due to the large number of apparent peaks present. Coelectrophoresis of protein solutions from ^{3}H-labeled controls and ^{14}C-labeled estrogen-treated uteri on a single gel, followed by determination of ^{14}C:^{3}H ratios, gives patterns which are more easily interpreted (see Figure 3).

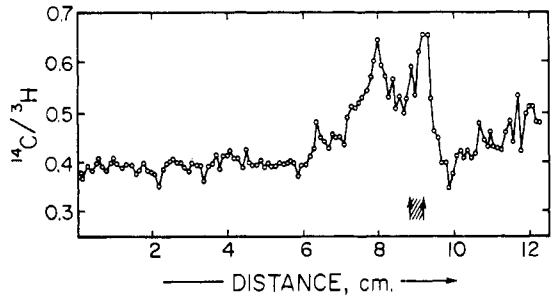

FIGURE 3: Electrophoretic analysis of estrogen-specific uterine proteins on longer gels. The 105,000*g* soluble proteins from an experiment similar to that described in Figure 2 were electrophoresed for 12 cm.

Measurement of Estrogen-Specific Proteins. The ^{14}C:^{3}H pattern from one such experiment is shown in Figure 2. Stimulated uterine proteins were obtained after 1-hr estrogen treatment and 1-hr incubation. Peaks of high ^{14}C:^{3}H ratio, which presumably indicate the estrogen-induced proteins, are present in both 20,000*g* and 105,000*g* supernatant fractions. A second, smaller peak, migrating faster than the main component appears occasionally. The highest ^{14}C:^{3}H ratios are found 1 hr after hormone treatment. Electrophoresis of labeled uterine proteins on longer gels results in the labeling pattern shown in Figure 3. Two major ^{14}C:^{3}H peaks are evident, migrating on either side of band 5 (arrows). Since longer gels result in decreased staining intensity, 6-cm gels were used in most subsequent studies.

Evidence that the radioactive peaks are not artifacts is derived from the experiment shown in Figure 4A in which hormone was not administered to either the ^{14}C group or the ^{3}H group and the expected straight line of ^{14}C:^{3}H ratios is obtained. Reversal of isotopes or use of amino acid-^{14}C mixture (Notides and Gorski, 1966) gives compar results.

The response of the estrogen-specific proteins to 17β-estradiol was measured after different periods of hormone stimulation (Figures 4 and 5). If animals are treated with hormone 2 hr prior to sacrifice, the electrophoretic pattern (Figure 4B) exhibits a single peak running behind band 5 as in Figure 2. However, two peaks are obtained when these proteins are electrophoresed for 12 cm, and they appear

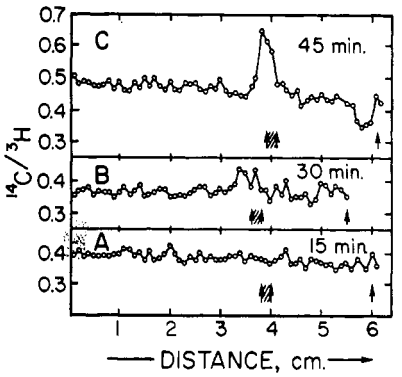

FIGURE 5: Earliest synthesis of estrogen-specific proteins after treatment with estradiol. Animals were divided into four groups; one group served as controls; the other three groups (A,B,C) received 5 μg of estradiol 15, 30, and 45 min, respectively, before sacrifice. Tissues from stimulated animals were incubated in medium containing leucine-^{14}C (84 mCi/mmole) for 20 min and the control group was labeled with leucine-*t* (1200 mCi/mmole) also for 20 min. An aliquot of each of the ^{14}C-labeled soluble-protein solutions was mixed with an equal volume of the ^{3}H-labeled soluble-protein solution before electrophoresis.

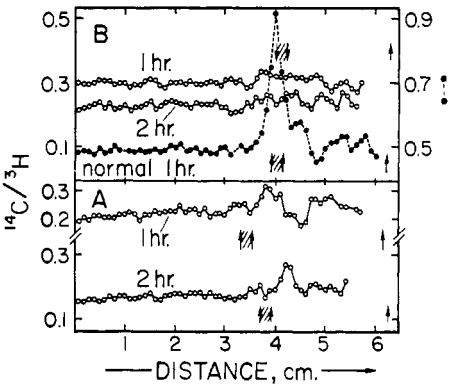

FIGURE 6: Effect of actinomycin D on synthesis of estrogen-specific proteins. Two groups of animals were treated with 200 μg of actinomycin D in saline, followed in 30 min by an injection of 5 μg of 17β-estradiol. One group was sacrificed 60 min after the hormone treatment. The other group was given an additional 100 μg of actinomycin D 60 min after the hormone and was sacrificed 60 min later. Both groups of uteri were labeled by incubation for 1 hr in HeLa medium containing leucine-^{14}C. Two groups of controls were used; one was treated with 200 μg of actinomycin D followed in 30 min by an injection of the hormone solvent. After 60 min the animals were sacrificed and the uterine proteins were labeled in leucine-*t* medium for 1 hr. The second control group was given saline solution instead of actinomycin D. Actinomycin D (17 μg/ml) was present in the incubation medium of all groups which were treated with the antibiotic *in vivo*. Figure 6A shows the ^{14}C:^{3}H pattern of the soluble proteins from both estradiol-treated groups (1 and 2 hr) electrophoresed with the proteins from the normal controls (no actinomycin treatment). Figure 6B shows the pattern from both stimulated groups electrophoresed with the actinomycin-treated controls. The solid circles (●--●) illustrate, for comparison, the usual 1-hr response to the hormone in the absence of actinomycin. The ^{14}C:^{3}H ratios for this are indicated on the right-hand side of Figure 6B.

on both sides of band 5. Treatment of the animals for 4 hr (Figure 4C) results in a much smaller peak, indicating that by this time the rate of synthesis of these proteins has decreased significantly relative to the other uterine proteins. Most of the soluble proteins migrate very slowly in this system, and a large part of the radioactivity incorporated in the 1-hr labeling period is in the slower moving proteins occupying the first 3 cm of the gels. This region contains no estrogen-specific peaks, but its ^{14}C:^{3}H values (Figure 4C,D) are increased considerably by 4 and 8 hr after estradiol injection. This increase in the value of the ^{14}C:^{3}H ratio reflects the increased total protein synthesis in the uterus due to estrogen (the "secondary response"). After treatment of the animals for 8 hr (Figure 4D) estrogen-specific protein peaks are not apparent although the total protein content and leucine-^{14}C uptake of the uterus increases significantly. The decreased ^{14}C:^{3}H ratios running ahead of band 5 are unexplained. It is not observed in studies with uteri from animals which had been stimulated for 36 hr before labeling. This region of the gel contains little stained material.

Increased synthesis of estrogen-specific proteins is detected 30 min after administration of estradiol (Figure 5). The detectable increase in the rate of synthesis of the other uterine-soluble proteins (Figure 5C) as noted by the increase in the ^{14}C:^{3}H ratio in the slowly migrating region, occurs with some delay.

In Vivo Labeling of Estrogen-Specific Proteins. *In vivo* administration of the isotopic amino acids in single or multiple doses to control animals and to animals injected 1 hr earlier with 17β-estradiol results in patterns of ^{14}C:^{3}H distribution which are similar to those obtained following *in vitro* labeling.

Effect of Actinomycin D on the Synthesis of Estrogen-Specific Proteins. Notides and Gorski (1966) reported that the synthesis of the estrogen-specific proteins detected in their system was inhibited only to about 50% by actinomycin D. Complete inhibition of the synthesis of these proteins was not observed even when actinomycin D was added to the incubation medium. Under our conditions, the synthesis of the estrogen-specific proteins is completely inhibited by actinomycin D, although the enhanced synthesis of a peak of "actinomycin-specific" protein is detected.

The ^{14}C:^{3}H patterns obtained from actinomycin-treated uteri which had been stimulated with estradiol for 1 and 2 hr and electrophoresed with controls which had not been treated with actinomycin do not contain the radioactive peaks characteristic of estrogen-induced proteins that usually appear near band 5. However, a new ^{14}C:^{3}H peak with greater mobility is evident (Figure 6A). That the appearance of this peak resulted from the use of actinomycin D is indicated by finding that when the ^{14}C-labeled uterine proteins from animals treated with both estrogen and actinomycin D are coelectrophoresed with ^{3}H-labeled uterine proteins from controls treated with actinomycin D alone, the "actinomycin-specific" peak seen in Figure 6A is absent (Figure 6B). The peak is present when ^{14}C-labeled proteins from animals treated only with actinomycin D are electrophoresed with ^{3}H-labeled proteins from untreated animals. These observations suggest that treatment of immature rats with actinomycin D results in the enhanced synthesis of a class of uterine proteins. The proximity of these proteins to the estrogen-specific peaks may account for the observations of Notides and Gorski (1966). De Angelo and Gorski (1969) have recently reported that synthesis of their estrogen-induced protein was blocked by actinomycin–nogalomycin treatment.

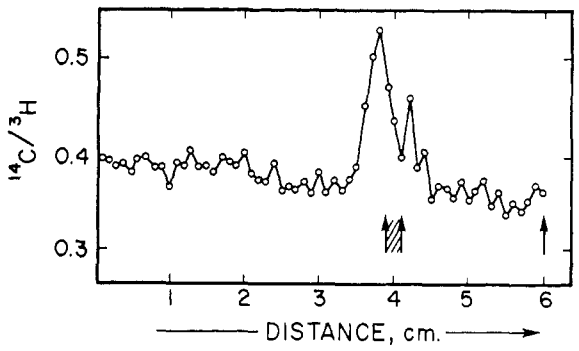

FIGURE 7: Effect of 17β-estradiol added *in vitro* on the synthesis of estrogen-specific proteins. Two groups of untreated animals were sacrificed and the uteri were removed. One group of uteri was preincubated for 1 hr in HeLa medium containing 2 μl of 100% ethanol (10 mM), followed by a 1-hr incubation with leucine-*t* added to the medium. The other group was preincubated for 1 hr in medium containing 2 μl of a solution of 17β-estradiol (10 mg/ml) in 100% ethanol, followed by a 1-hr incubation with leucine-^{14}C added to the medium. The soluble fractions of each group were mixed and electrophoresed as usual.

Effect of 17β-Estradiol Added in Vitro on the Synthesis of Estrogen-Specific Proteins. The stimulation of synthesis of the estrogen-specific uterine proteins by estradiol is detected following the *in vitro* addition of the hormone to the culture medium containing uteri from untreated immature rats (Figure 7), but the effect is considerably less than that observed after *in vivo* hormone administration. The presence of ethanol in the medium (10 mM) has no apparent effect on the rate of amino acid incorporation into protein. The value of the $^{14}C:^{3}H$ ratio of the slower moving proteins remains unchanged even after extended periods of *in vitro* hormone stimulation. These results have been repeated several times with 17β-estradiol, but estriol, a less active estrogen, produces no detectable stimulation.

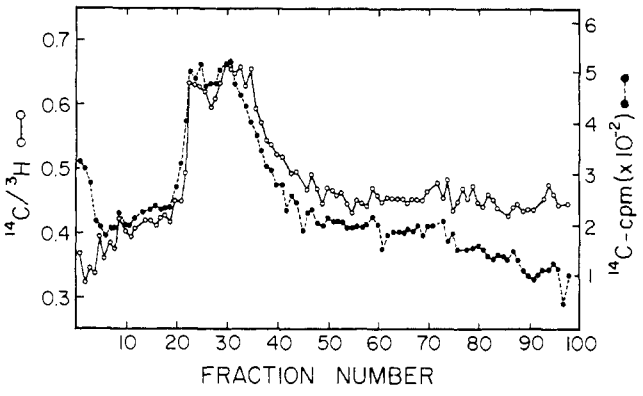

FIGURE 8: Preparative polyacrylamide gel electrophoresis of estrogen-specific proteins. Two groups of immature rats were used. One group was injected with estradiol 1 hr before removing and incubating the uteri in the leucine-^{14}C medium; the control group was labeled with leucine-*t*. Tissues were stored at −80° until enough had been accumulated (51 in each group). The uteri were homogenized in 0.015 M KCl, centrifuged, and the mixed 105,000*g* fractions (5 ml) were applied to the preparative electrophoresis apparatus and electrophoresed at 300 V and 50 mA for 17 hr. Fractions of 4.5 ml were collected and 0.5-ml aliquots were used for protein analysis by the Lowry method and for $^{14}C:^{3}H$ values of the material obtained by precipitation with 10% trichloroacetic acid onto Millipore filters and washing with 5% trichloroacetic acid. The $^{14}C:^{3}H$ ratios and ^{14}C-radioactivity counts per minute per 0.5-ml aliquot are presented. A total of 250 fractions were examined.

Preparative Gel Electrophoresis. Because of their high electrophoretic mobility, the estrogen-specific proteins can be purified by preparative gel electrophoresis. The pattern of radioactivity separated by the preparative gel electrophoresis apparatus is shown in Figure 8. As was observed with analytical gels, the $^{14}C:^{3}H$ pattern is resolved into a double peak which coincides with the maximum of total radioactivity. There are no apparent protein peaks in the elution profile due, probably, to the heterogeneity of the protein solutions and to the lower resolution inherent in the preparative relative to analytical polyacrylamide gels.

Fractions 23 to 38 contain approximately 1.2 mg of protein, or 2.3% of that applied to the gel. The pooled fractions were concentrated by membrane filtration to 1.0 ml which contained 3×10^{4} cpm of acid-insoluble ^{14}C with a $^{14}C:^{3}H$ ratio of 0.63. This material was further purified by electrofocusing using a pH gradient of 3–5 on an LKB 8101 electrofocusing column. A series of heterogeneous peaks was observed with $^{14}C:^{3}H$ ratios of 0.95 to 1.10 within the pH range 3.50–4.00 that corresponded to peaks of total radioactivity.

Discussion

Polyacrylamide gel electrophoresis of a mixture of ^{14}C-labeled proteins from estrogen-stimulated uteri and ^{3}H-labeled proteins from untreated uteri led to the detection of protein fractions characterized by elevated $^{14}C:^{3}H$ ratios. The synthesis of these estrogen-specific proteins was enhanced by the hormone treatment before detectable changes occurred in the rate of synthesis of other uterine proteins (the "secondary response"). Similar estrogen-specific proteins were detected earlier by Notides and Gorski (1966) using starch gel electrophoresis and single isotopes. These proteins comprise a heterogeneous group with pI values in the range of 3.5 to 4.0 as measured by electrofocusing. Although they are present in the 105,000*g* supernatant fractions the possibility is not excluded that they were released from a subcellular fraction during homogenization.

Comparison of the patterns of radioactivity obtained when the double-labeled mixture of proteins from hormone-stimulated and control animals is electrophoresed on starch gel as described by Notides and Gorski (1966) and on polyacrylamide gel indicates that the estrogen-specific proteins observed in the two laboratories are probably the same even though their electrophoretic mobilities in the two media are somewhat different.

The incorporation of amino acids into estrogen-specific proteins at various times after treatment with estradiol indicates that their synthesis occurs prior to the synthesis of most of the uterine proteins, and suggests that they play an early role in the mechanism of action of the hormone. It is possible that the estrogen-specific proteins are synthesized in response to estradiol to retain larger amounts of the hormone within the cells (Jensen and Jacobson, 1962). Alternately, they may be key enzymes, located in critical regulatory areas of intermediary metabolism.

The synthesis of estrogen-specific proteins is completely

inhibited by treatment with actinomycin D in contrast to the partial inhibition found by Notides and Gorski (1966). Surprisingly, actinomycin D evokes the synthesis of an "actinomycin-specific" uterine protein with electrophoretic mobility similar to that of the estrogen-specific proteins.

The synthesis of the estrogen-specific proteins is also detected after *in vitro* treatment of isolated uteri with 17β-estradiol added to the incubation medium. Under these conditions the "secondary estrogen response" is not detected by prolonged treatment with hormone.

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Interactions between Corticosteroids and Histones*

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ABSTRACT: This project was designed to test the validity of previous work which concluded that cortisol reacts with histones. In this paper, it is reported that the interaction of cortisol-1,2-*t* and histone was due to the presence of 21-dehydrocortisol-1,2-*t* in the steroid preparations. This conclusion was based on the following observations: Cortisol-1,2-*t* and 21-dehydrocortisol, though reacting with all histone fractions, bound most extensively to arginine-rich histone. Blocking of arginyl residues of histones by glyoxalation greatly diminished binding of 21-dehydrocortisol-1,2-*t* and eliminated binding of cortisol-1,2-*t*. It was concluded that unblocked guanidino residues were essential for the reaction of steroid with histone. Blocking of lysyl residues alone by maleylation had only a small effect on the reaction of either steroid with histone. In model systems using solvent partition analysis, cortisol did not react with any free amino acid, while 21-dehydrocortisol reacted with all free amino acids except proline and hydroxyproline. Net reaction of 21-dehydrocortisol with arginine and histidine was four to five times greater than with most monocarboxylic–monoamino acids, and two times greater than with lysine. Arginine and 21-dehydrocortisol combined in equimolar ratio and irreversibly. Lysine and other amino acids were bound reversibly and less extensively. Preferential reaction of 21-dehydrocortisol with arginyl residues of histone was demonstrated by direct amino acid analysis of the stable steroid–histone complex after acid hydrolysis. This finding was consistent with the behavior of the steroids with modified histones and with free amino acids. 21-Dehydrocortisol-1,2-*t* was a consistent contaminant in all cortisol-1,2-*t* preparations.

It was concluded that binding of corticosteroids to histones occurred only after oxidation of the cortisol ketol side chain and that the reaction involved covalent interactions with the terminal residues of the basic amino acids, arginine and, to a lesser extent, lysine.

Considerable effort has been expended in recent years in attempts to explain how the levels and specific properties of proteins in cells of multicellular organisms are controlled. In 1950, Stedman and Stedman proposed that histones, found abundantly in the somatic nuclei of eukaryotes, could exert such control at the level of gene expression, possibly (as others have suggested (Huang and Bonner, 1962)) by reacting with DNA and thereby diminishing transcriptional activity. It is not yet clear if histones influence RNA synthesis in nuclei by performing such a "repressor-like" role, or whether their main function is to impart rigidity and stability to the intranuclear structures (Mirsky *et al.*, 1968; Chalkley and Jensen, 1968). That histones do inhibit the action of DNA-dependent RNA polymerase in model systems is indisputable. The mechanism *in vitro* probably involves neutralization of the negatively charged groups of DNA by the lysyl and arginyl residues of basic histones. These electrostatic interactions can be diminished if the charge on the histone is decreased by enzymic phosphoryla-

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