Estrogen Control of Carbohydrate Metabolism in the Rat Uterus: Pathways of Glucose Metabolism

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ABSTRACT. The utilization of glucose-1-^{14}C and glucose-6-^{14}C by uterine segments removed from ovariectomized rats at various times after treatment with 5.0 μg of 17β-estradiol has been evaluated. Activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were also determined in these tissues. While total activity of both enzymes (U/uterus) remained constant for 6 hr after estrogen administration, a 2-fold increase occurred by the 12th hr. Specific activity (U/mg protein) of both enzymes decreased during the first 6 hr. Thus, induction of these enzymes is not among the very early uterine responses to 17β-estradiol. Formation of CO_2 *in vitro* from carbon atoms 1 and 6 of glucose by uterine segments 2 hr after estradiol treatment is greater than that by control and 6-hr post-estrogen-treated segments. Between 6 and 24 hr the increased CO_2 production from glucose is primarily from carbon atom 1. The ratio of carbon 1 to carbon 6 incorporated into CO_2 increases linearly with time after estrogen administration. These data suggest 2 estrogen-sensitive mechanisms for control of glucose metabolism. Incorporation of carbon atoms 1 and 6 of glucose into lipid is equal and increases linearly with time. Incorporation of carbon atoms 1 and 6 of glucose into protein occurs at similar rates for 6 hr after estrogen administration. Thereafter a significant increase in incorporation of carbon 1 over carbon 6 is observed. The incorporation of carbon atom 1 of glucose into RNA parallels its incorporation into CO_2. The same is true of carbon atom 6. (*Endocrinology* **78:** 1205, 1966)

ADMINISTRATION of an estrogenic substance has been previously shown to increase the biosynthesis of protein, lipid, glycogen, ribonucleic acids and nucleic acids in the uterus (1–9). Considering the wide ramifications to be expected from the stimulation of these biosynthetic events, it is not surprising that such processes as glucose uptake and oxidation are also stimulated (10, 11).

This investigation was designed to examine the relationship between enzyme activity and the activity of certain metabolic pathways in the rat uterus following a single intravenous injection of 17β-estradiol. The enzymes studied were G6PD[3] and 6PGD. The relative rates of glucose utilization via the Embden-Meyerhof, or glycolytic, pathway and the hexose monophosphate shunt pathway in rat uterine segments were assessed by following the formation of $^{14}CO_2$ from glucose-1-^{14}C and glucose-6-^{14}C under *in vitro* conditions. The rates of incorporation of the two glucose carbon atoms into lipid, protein and RNA of the uterine segments under the *in vitro* incubation conditions are also reported.

Materials and Methods

Female rats of the Holtzman strain weighing 180–200 g were ovariectomized by the dorsal approach under ether anesthesia at least 4 weeks prior to use. The animals were killed by decapitation 2, 6, 12 and 24 hr after the tail vein injection of 5.0 μg of 17β-estradiol in 0.5 ml of 5% ethanol in isotonic saline. Control animals received only the vehicle immediately prior to their sacrifice.

Tissue preparation. Uteri from 3 similarly treated animals were placed in cold Robinson's

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[3] Abbreviations used in this manuscript are: C-1, carbon atom 1 of glucose; C-6, carbon atom 6 of glucose; C-1/C-6, ratio of $^{14}CO_2$ from glucose-1-^{14}C to $^{14}CO_2$ from glucose-6-^{14}C; G6PD, glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.49); 6PGD, 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ oxidoreductase, EC 1.1.1.44).

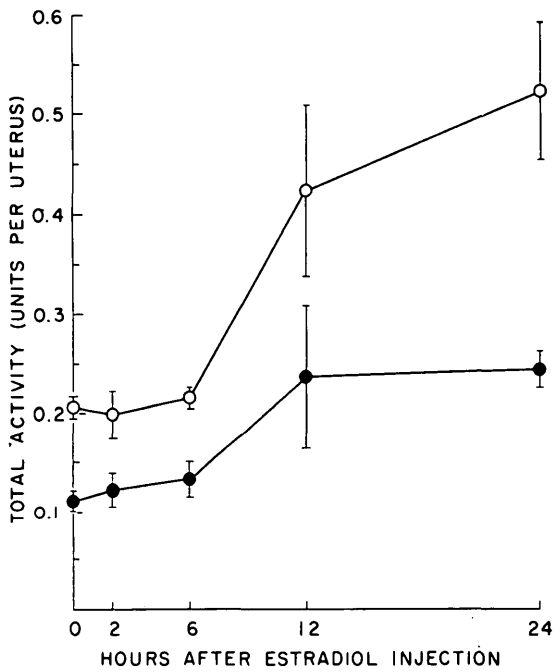

FIG. 1. Effect of estradiol on the total activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the rat uterus. Units of G6PD (open circles) and 6PGD (closed circles) expressed as means ±SE.

(12) medium immediately after removal. Each uterus was trimmed of fat and connective tissue. The 2 horns were separated at the body of the uterus, and each horn was opened longitudinally with scissors. A section from the long axis of each uterine horn was removed and pooled for enzyme assays, and the remaining portion was cut into 4 equal segments. Segments from the 3 animals were pooled and assigned randomly to 2 matched groups for incubation with C-1 or C-6 labeled glucose. The wet weight of each group of segments was used to determine the percentage of a total uterus represented in each group. Each incubation flask contained segments equivalent to $100 \pm 15\%$ of a whole uterus and the results of each evaluation parameter were corrected for these deviations.

Enzyme assay. The longitudinal uterine sections were homogenized in 2.0 ml of Robinson's medium using an all-glass homogenizer. The homogenate was centrifuged at $30{,}000 \times g$ for 15 min in a refrigerated centrifuge. The supernatant was decanted into a precooled tube and 0.1 ml aliquots were subjected to enzyme assay. G6PD and 6PGD activities were measured by the procedures of Glock and McLean (13) with some modifications. The reaction mixture contained 100 μmoles of (Tris) hydroxymethylaminomethane-HCl buffered at pH 7.5, 20 μmoles of $MgCl_2$, 1.0 μmole of $NADP^+$, 1.0 μmole of 6-phospho-D-gluconate with and without 1.0 μmole of D-glucose-6-phosphate in a final volume of 2.9 ml. The last 3 reagents were obtained from the Sigma Chemical Co. After the addition of 0.1 ml of the uterine supernatant, the reaction was followed at 340 mμ in a Beckman DU recording spectrophotometer at a temperature of 37 C. Control assays (without any substrate) demonstrated no reduction of $NADP^+$. Initial velocity of the reaction mixture containing only 6-phosphogluconate was taken as 6PGD activity. This value subtracted from the initial velocity of the reaction mixture containing both substrates was taken as G6PD activity. The protein content of the supernatant was determined by the method of Lowry *et al.* (14). Enzyme activities are expressed as U/uterus and U/mg of protein. A unit is defined as that amount of enzyme capable of reducing 1.0 μmole of $NADP^+$/min under the conditions of the assay. This definition conforms to the recommendations of the Commission on Enzymes of the International Union of Biochemistry (15).

Incubation of uterine segments. Uterine segments were incubated for 2 hr at 37 C in 2.0 ml of Robinson's medium containing 2 mg (0.25 μc/mg) of either glucose-1-^{14}C or glucose-6-^{14}C (Nuclear-Chicago). Radioactive CO_2 was measured by the procedure of Cuppy and Crevasse (16). Lipid, RNA and protein fractions were isolated from the segments after incubation as described by Gorski and Nicolette (17, 18). Lipid and protein fractions were dissolved in 1.0 ml of absolute ethanol and 1.0 ml of hyamine hydroxide, respectively. A toluene-phosphor solution was added, and the radioactivity was quantitated in a liquid scintillation counter using an internal standard. RNA fractions were counted in a windowless gas-flow counter by plating an aliquot of the RNA extract on lens paper discs and counting by an infinite thick layer technique. RNA data are corrected to cpm obtained by the liquid scintillation method (cpm represent 85% of dpm) to allow direct comparison. Protein was quantitated by the method of Lowry *et al.* (14). Lipid was evaluated by measuring total phosphate in the lipid fraction using the method of Bartlett (19). RNA was measured at 260 mμ and converted to a weight basis using an absorbancy coefficient of 21.4 cm^2mg^{-1} (see ref. 20). The results are expressed as cpm/uterus in CO_2, protein, lipid and RNA and cpm/U of protein (mg), lipid (μmoles phosphate) and RNA (mg).

The data are presented graphically as a mean

±standard error of the mean with each mean representing 6 groups of 3 animals/group. The mean and SE for derived parameters were calculated from the individually derived values for each matched incubation set. The same animals are represented in each parameter evaluated within a given time period of estrogen stimulation. Statistical significance of results was determined using methods described by Snedecor (21). The various results were tested against 3 levels of probability: $p < .01$, highly significant; $p < .05$, significant; and $p < .10$, approaching significance.

Results

Estradiol-induced enzyme synthesis. The total activity of G6PD and 6PGD in the rat uterus remains relatively constant for the first six hours following a single intravenous injection of 17β-estradiol (Fig. 1). Between six and 12 hours there is a 2-fold increase in total activity of both enzymes.

The specific activity of G6PD in the uterus is decreased in an almost linear manner during the first six hours of estrogen stimulation (Fig. 2). The decrease is highly significant at $p < .01$. Similarly, it appears that 6PGD is decreased at six hours; however, the results are less dramatic and are substantiated only by the trend of the data. By the twelfth hour the specific activity of G6PD has again increased back to a level comparable to that of the control.

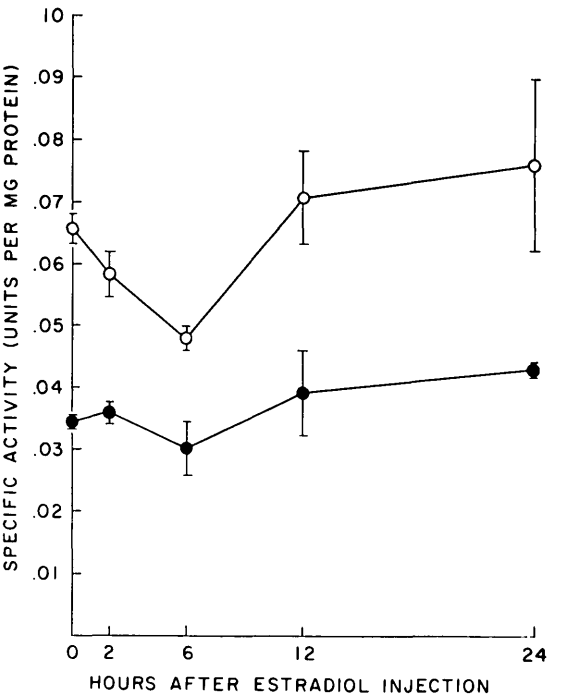

FIG. 2. Effect of estradiol on the specific activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the rat uterus. Units of G6PD (open circles) and 6PGD (closed circles) per mg of protein are expressed as means ±SE.

Formation of CO_2 from specifically labeled ^{14}C-glucose. There is a significant increase in the rate of formation of $^{14}CO_2$ from both glucose-1-^{14}C and glucose-6-^{14}C in the uterus of the castrated rat two hours after estrogen treatment (Fig. 3). Six hours after treatment the rate of formation of CO_2 from either carbon of glucose by the surviving uterine segments is not significantly different from that of the nonstimulated uterus. The increase in rate of $^{14}CO_2$ formation from glucose by uterine segments 12 and 24 hours after estradiol treatment occurs primarily by stimulation of the rate of removal of C-1 from glucose.

The ratio of $^{14}CO_2$ formed from glucose-1-^{14}C to that from glucose-6-^{14}C was observed to increase with respect to time after estradiol injection (Fig. 4). Statistically, the rate of increase of this ratio was observed to be both positive and linear at $p < .01$.

Enzyme activity and pathway utilization. The total activity of G6PD in the uterus and the rate of formation of $^{14}CO_2$ from glucose-1-^{14}C are correlated at $r = .815$ when all time intervals after estradiol injection are considered. When the second hour values are deleted and the correlation coefficient is again calculated, the value of $r = .900$ is obtained. A test of the statistical significance of these associations indicates a positive association at $p < .01$. When cpm in the $^{14}CO_2$ from glucose-1-^{14}C is regressed on the activity of G6PD, a regression coefficient of 14,100 cpm per two-hour incubation is obtained per unit of G6PD in the uterus.

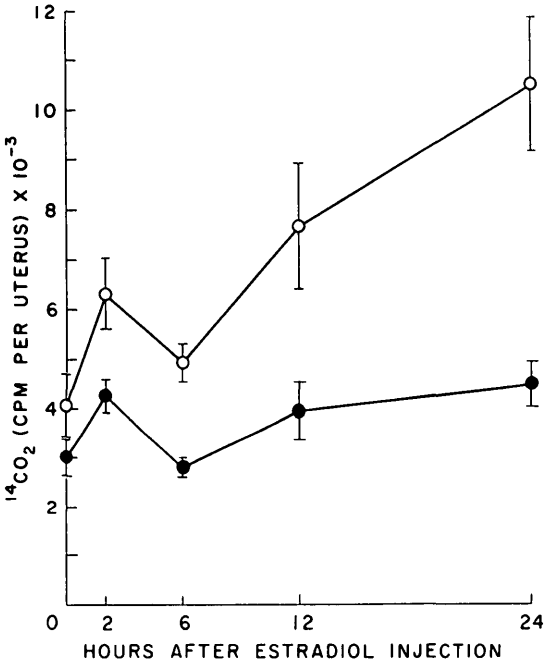

FIG. 3. Effect of estradiol on the rate of incorporation of labeled glucose into $^{14}CO_2$ by rat uterine segments. Amounts of $^{14}CO_2$ produced in a 2-hr incubation *in vitro* from glucose-1-^{14}C (open circles) and glucose-6-^{14}C (closed circles) expressed as means ±SE.

Synthesis of lipid from glucose. The rate of synthesis of lipid from glucose as a carbon source in surviving uterine segments from the castrated rat increases in a linear manner with respect to time after estradiol injection (Fig. 5). Statistically, there is no difference in the rate of incorporation of C-1 and C-6 from glucose into uterine lipids.

The specific activity of the lipid fractions (as evaluated by the parameter of lipid phosphate) isolated from the uterine segments after incubation in labeled glucose undergoes a 50% increase during the first two hours after estradiol injection. After the second hour the rate of increase is considerably less, due to the increasing lipid content of the uterus.

Synthesis of protein from glucose. The rate of incorporation of C-1 and C-6 from glucose into the protein fraction of surviving uterine segments is similar for six hours following estradiol injection (Fig. 6). During this six-hour period the rate of incorporation of both carbon atoms of glucose into protein increases by 70% over that of the control. Twelve hours after estradiol treatment C-1 of glucose is incorporated into uterine protein at a greater rate than C-6 ($p < .01$).

The specific activity of the protein in the uterine segment after incubation with labeled glucose presents a biphasic pattern with respect to time after estradiol injection. At two hours after estradiol administration, protein specific activity in segments incubated with both C-1 and C-6 labeled glucoses are increased 70% above pretreatment levels. Six-hour specific activities are only 45% above pretreatment levels. At later periods specific activity with C-1 labeled glucose returns to 70% above while C-6 specific activities remain at 45% above pretreatment levels. The total protein content of the uterus was found to stay relatively constant for two hours, after which the amount increased at a rather constant rate to the twelfth hour.

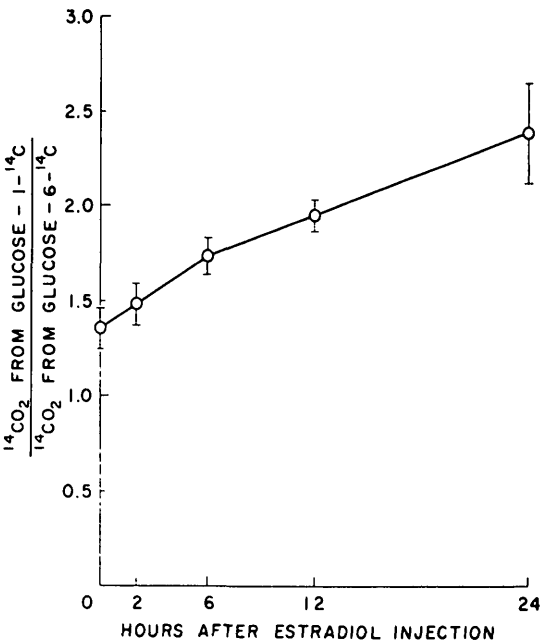

FIG. 4. Effect of estradiol on the ratio of $^{14}CO_2$ produced per uterus from glucose-1-^{14}C to $^{14}CO_2$ produced from glucose-6-^{14}C. CO_2 produced in a 2-hr incubation *in vitro* by rat uterine segments. Values are expressed as means ±SE.

Synthesis of RNA from glucose. The rate of incorporation of both C-1 and C-6 of glucose into RNA is increased after two hours of estradiol stimulation (Fig. 7). Carbon atom 1 of glucose is consistently incorporated into the RNA fraction at a rate greater than that of C-6. The specific activity of the RNA fraction very closely parallels the total activity, as expected from the nearly constant amount of RNA in the uterus during the period studied.

Relationship of $^{14}CO_2$ produced to ^{14}C-RNA formed. A comparison of the pattern of $^{14}CO_2$ formation to the labeling pattern of RNA suggests a considerable similarity. A correlation coefficient between $^{14}CO_2$ from glucose-1-^{14}C and the total label incorporated into the corresponding RNA fraction of $r = .771$ was obtained. A similar calculation using $^{14}CO_2$ from glucose-6-^{14}C yielded a value of $r = .602$. These associations were highly significant ($p < .01$).

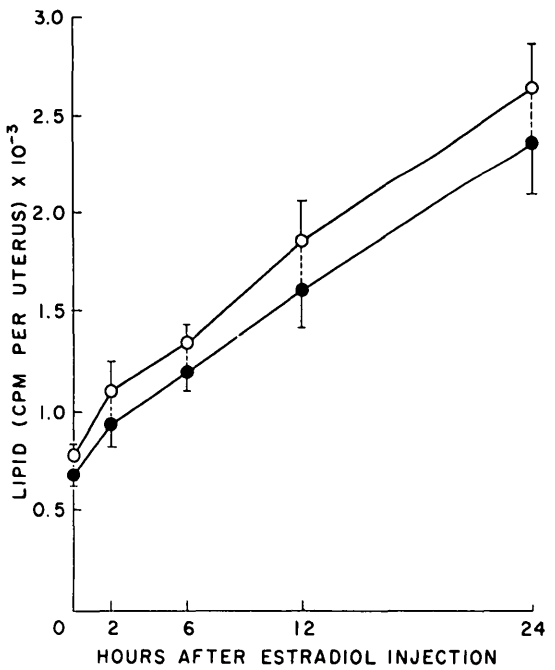

FIG. 5. Effect of estradiol on the rate of incorporation of labeled glucose into the total lipid fraction of a rat uterus. Radioactivities of lipid fractions from uterine segments incubated for 2 hr *in vitro* with glucose-1-^{14}C (open circles) and glucose-6-^{14}C (closed circles) expressed as means ±SE.

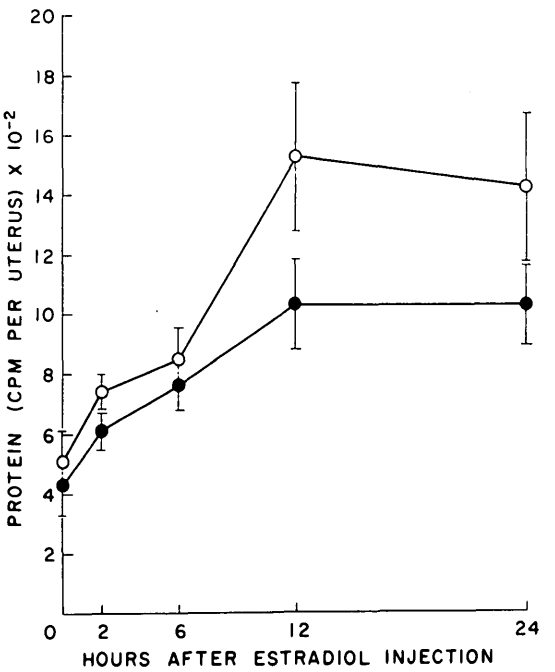

FIG. 6. Effect of estradiol on the rate of incorporation of labeled glucose into the total protein fraction of rat uterus. Radioactivities of protein fractions from uterine segments incubated for 2 hr *in vitro* with glucose-1-^{14}C (open circles) and glucose-6-^{14}C (closed circles) expressed as means ±SE.

The differences in correlation coefficients suggested some dissimilarity in the labeling patterns between the two forms of labeled glucose. A regression analysis yielded regression coefficients of 0.293 and 0.342 for the comparisons of cpm in RNA to cpm in CO_2 from glucose-1-^{14}C and glucose-6-^{14}C, respectively. These regression coefficients were different at $p < .01$.

Discussion

While a single, unified explanation of the mechanism of estrogen action is not yet at hand, there is a growing body of information which implicates regulation of protein synthesis as this basic mechanism. Because the hexose monophosphate shunt system provides ribose and reduced $NADP^+$ and because increases in G6PD and 6PGD activities in uterus after estrogen administration have been observed (22, 23), the role of this "system" in the

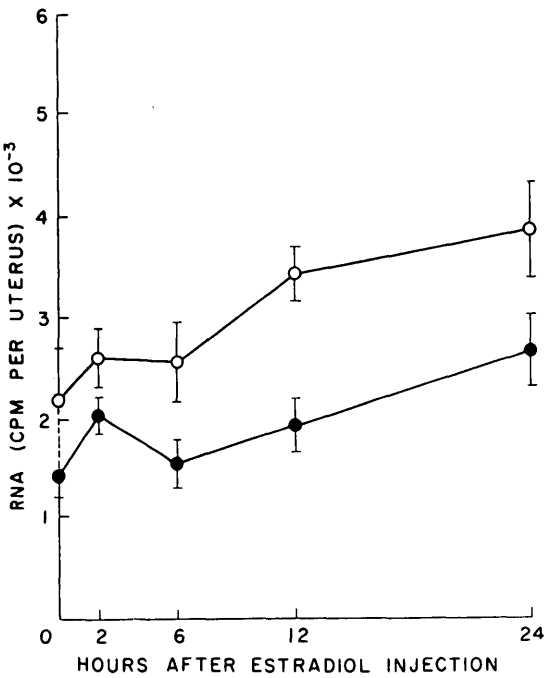

FIG. 7. Effect of estradiol on the rate of incorporation of labeled glucose into the total RNA fraction of rat uterus. Radioactivities of RNA fractions from uterine segments incubated for 2 hr *in vitro* with glucose-1-^{14}C (open circles) and glucose-6-^{14}C (closed circles) expressed as means ±SE.

early, primary response to estrogen requires evaluation.

In the interpretation of the ^{14}C incorporation data presented above, it must be remembered that total radioactivity in a product isolated from an entire organ: a) represents a specifically labeled carbon atom of glucose that arrives at its destination during a two-hour *in vitro* incubation period, and b) gives an approximation of all the rates (or at least that of the limiting step) from the labeled precursors to the product studied if no great changes in pool sizes of the precursor or intermediates occur. Specific activity is a function of total activity plus the added factor of the proportionality of the product either present at the outset of the incubation or arising from pathways not involving the labeled precursor to that which is synthesized from labeled precursors during the incubation period. Since the amount of a product in an organ may or may not vary with certain treatments, when specific activity parameters are used to describe organ function they must be interpreted very carefully.

Enzyme synthesis in the uterus. Comparison of Fig. 1–7 clearly indicates that increased synthesis of G6PD and 6PGD does not occur as early as many other uterine responses to estrogen. Scott and Lisi (22) also observed decreased specific activity of G6PD in the rat (U/mg protein) during the first six hours after estrogen administration and ascribed it to uptake of serum albumin by uterine tissue. In this study an early reduction of G6PD specific activity approximating 30% was observed. That this increase in non-G6PD protein may result wholly or in part from synthesis rather than uptake is suggested by the observation that the rate of glucose incorporation into uterine protein is increased to 170% of controls six hours after estrogen administration. Nevertheless, it is obvious that no increase in G6PD or 6PGD activity occurs within the first six hours after estrogen administration, while a previous observation on RNA polymerase activity (24) shows it to be clearly elevated within one hour.

On the other hand, the activities of these enzymes are increased at 12 and 24 hours after estrogen administration. Whether these increases represent a direct, specific estrogen effect or a substrate type induction by $NADP^+$ [suggested by Tepperman and Branch (25) to be responsible for induction of $NADP^+$-linked dehydrogenases in rat liver] or glucose-6-phosphate remains to be seen. Although no data are available on glucose-6-phosphate concentrations in uterus following estrogen administration, Spaziani (26) has shown that estradiol accelerates transport of hexoses into the uterus within two to four hours after administration.

Pathways of glucose utilization. Within the limitations in the use of C-1 and C-6 labeled glucose to evaluate the rates of the glycolytic and hexose monophosphate path-

ways (27), it appears that the majority of the later estrogen stimulation (*i.e.*, after 6 hr) of glucose utilization in the uterus occurs through the hexose monophosphate shunt. The increased production of CO_2 from C-1 and C-6 of glucose two hours after estrogen stimulation is in accord with the observations of Nicolette and Gorski (11) using randomly labeled glucose. The absence of increased production of CO_2 from C-1 labeled glucose six hours after estrogen treatment is in accord with the observations of Mueller and Herranen (28).

It is of interest to note the linear increase in the C-1/C-6 ratio of CO_2 formation from glucose carbons with respect to time following estradiol administration. Unlike total CO_2 formed, the ratio does not deviate from linearity at two hours post-estrogen treatment. These observations suggest that at least *two independent factors* control the utilization of glucose by uterine tissue.

G6PD activity and CO_2 formation from glucose-1-^{14}C. It appears that variation in shunt activity may clearly be broken up into two phases: a) an early phase, lasting six hours and characterized by increased shunt activity with no increase in G6PD and 6PGD activities, and b) a later phase which begins sometime between six and 12 hours and is characterized by increasing enzyme activities closely paralleling hexose monophosphate shunt activity.

The high degree of correlation between G6PD activity and the formation of $^{14}CO_2$ from glucose-1-^{14}C suggests either that one is dependent on the other or that the two factors are linked by a common intermediate controlling factor. The regression coefficient of $^{14}CO_2$ from glucose-1-^{14}C on the activity of G6PD suggests that only about one thousandth of the enzyme capacity (as determined under conditions of saturation with substrate and cofactor) is utilized in the observed *in vitro* activity of the hexose monophosphate shunt. While it is appealing to consider changes in enzyme activity as the cause of increased hexose monophosphate shunt activity, other factors (substrates and inhibitors) might be responsible, particularly in the early phase of the estrogen response, since the C-1/C-6 ratio increases before enzyme levels increase. The only factor other than the C-1/C-6 ratio in $^{14}CO_2$ observed in this study which exhibits a linear increase with time after estrogen injection is the rate of lipid synthesis from glucose carbon precursors. These two factors were found to be correlated at $r = .701$, which indicates an association of the two factors at $p < .01$.

Lipid synthesis is accompanied by conversion of NADPH to $NADP^+$. This increase in $NADP^+$ could activate the hexose monophosphate shunt pathway even without a change in the activities of G6PD or 6PGD. An increase in $NADP^+$ could also arise from activation of a transhydrogenase which transfers hydrogen from NADPH to $NADP^+$. Such an estrogen-sensitive transhydrogenase system is present in human placenta (29, 30) and human uterus but is lacking in rat uterus (32). Thus, $NADP^+$ may be a common denominator in three closely linked factors: level of G6PD, hexose monophosphate shunt activity, and lipid synthesis.

Irrespective of mechanism or phase of the response, it is apparent that total hexose monophosphate shunt activity and the percentage of glucose being metabolized via this pathway in the uterus increase following estrogen stimulation.

Lipid synthesis from glucose precursors. The rate of synthesis of lipids from glucose by uterine segments after estrogen stimulation follows the same linear pattern that was observed by Aizawa and Mueller (7) for the rate of incorporation of acetate-1-^{14}C into fatty acids. The similarity in the rates of incorporation of C-1 and C-6 of glucose into lipid suggests both may be metabolized via the same pathways, as would be the case if the formation of the lipid precursors occurred primarily through the Embden-Meyerhof pathway.

Incorporation of glucose into uterine protein. The incorporation of C-1 and C-6 into

uterine protein occurs at a similar rate for six hours post-estrogen treatment. After 12 hours there is a significant increase in the rate of incorporation of C-1 over C-6. Since at this time C-1 is removed as CO_2 at a greater rate than C-6, it would be expected that the rate of incorporation of C-6 into protein should either be equal to or greater than C-1 unless the increased incorporation of C-1 into protein may be through CO_2 fixation. Jervell *et al.* (9) have observed an increased rate of incorporation of $^{14}CO_2$ into uterine aspartate and glutamate 12, but not six hours after estrogen stimulation. Thus, it appears that between six and 12 hours a marked increase in the capacity for CO_2 fixation into amino acids occurs. Subject to the assumption that pool sizes of aspartate and glutamate do not change markedly in estrogen-stimulated uteri, the 5-fold greater specific activity of aspartate over glutamate observed by these investigators suggests that aspartate and possibly its α-keto acid, oxaloacetate, may be close to the site of CO_2 fixation.

Glucose incorporation into uterine RNA. Under the conditions of the present experiments, the incorporation of a glucose carbon into RNA appears to be associated with the formation of $^{14}CO_2$ from the same glucose carbon. Since glucose C-1 incorporation into RNA and CO_2 is greater than C-6 at all times, one is led to suspect that CO_2 fixation into RNA precursors (probably the purine and pyrimidine bases) may be largely responsible for the labeling.

The regression coefficients for cpm in the CO_2 to cpm in RNA suggest that, in relation to glucose-1-^{14}C, the label from glucose-6-^{14}C gets into RNA by an additional pathway. Possibly this is no more than the ribose itself, which, if it arises from glucose-1-^{14}C via the hexose monophosphate shunt, would be free of label.

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