to 70 days later their immunological response to 2 different antigens, sheep red blood cells and *S. typhosa* H, was assessed. The hormone treated rat produced less antibodies in response to both these antigenic challenges than did control animals.

We wish to thank Dr. Ronald M. Wood, California Dept. of Public Health, Berkeley, for a generous gift of *S. typhosa* H antigen.

1. Smith, D. W., Blizzard, R. M., Wilkens, L. W., Pediatrics, 1957, v9, 1011.

2. Eayrs, J. T., J. Endocrinol., 1961, v22, 409.

3. Barraclough, C. A., in Nalbandov, A. V. (ed.), Advances in Neuroendocrinol., Univ. of Illinois Press, Urbana, 1963, 224.

4. Schlesinger, M., Mark, R., Science, 1964, v143, 965.

5. Howard E., J. Neurochem., 1965, v12, 181.

6. Schapiro, S., Proc. Soc. Exp. Biol. & Med., 1965, v120, 171.

7. Fachet, J., Palkovits, M., Vallent, K., Stark, E., Acta Endocrinol., 1966, v51, 71.

8. Miller, J. F. A. P., Proc. Roy. Soc. London, 1962, v156, 4151.

9. Osoba, D., Miller, J. F. A. P., Nature, 1963, v199, 653.

10. Jankovic, B. D., Waksman, R. H., Arnason, B. G., Exp. Med., 1962, v116, 159.

11. Schapiro, S., Geller, E., Eiduson, S., Proc. Soc. Exp. Biol. and Med., 1962, v109, 937.

Received September 29, 1966. P.S.E.B.M., 1967, v124.

Location of the Estrogen Effect on Uterine Glucose Metabolism.* (31843)

DONALD E. SMITH† (Introduced by F. R. Steggerda)
Ohio Wesleyan University, Delaware, Ohio

One of the rapid and most pronounced responses of uterine target tissue to estrogen is an acceleration of the pathways of glucose metabolism. Examples of this effect, including glucose utilization and glycogen synthesis, have been reported by several workers (1,2,3). Recently, Nicolette and Gorski(4) found a relationship between increased glucose metabolism and other estrogen effects when they showed that estradiol-17β increased the incorporation of radioactivity from ^{14}C-labelled glucose into uterine lipid, CO_2, RNA, and protein. These studies imply that some early step in uterine glucose metabolism is involved in the tissue response. Characteristics of this response suggest that the estrogen effect could be located: (1) in the Embden-Meyerhoff pathway, (2) the pentose phosphate cycle, or (3) some step common to both these pathways.

The experiments described here have utilized measurements of $^{14}CO_2$ production and radioactivity incorporation into lipid from labelled substrates to study the pathways in glucose metabolism which are influenced by estrogen. These studies reveal that the estrogen-sensitive step is probably located prior to the formation of glucose-6-phosphate from glucose. This indicates that either the transport step or the phosphorylation step is the site of early estrogen action on uterine glucose metabolism.

Materials and methods. Immature, female, Holtzman rats (21-24 days old) were injected intraperitoneally with 5 μg of estradiol-17β dissolved in .5 ml of 1% ethanol in 0.15 M NaCl. Control animals received only the injection vehicle. Animals were killed 2 hours after treatment, uteri were excised, weighed, and transferred immediately to ice-cold incubation medium in 25 ml Erlenmeyer flasks. Uteria were incubated in an atmosphere of 95% O_2-5% CO_2 at 37°C in 2.0 ml of Eagle's tissue culture medium HeLa (Difco). This medium contains glucose (90 mg%) but does not contain pyruvate. The incubation medium contained 0.1 μc/ml of glucose-1-^{14}C (3.14 mc/m mole) or glucose-6-^{14}C (3.56 mc/m mole) as substrate. Where pyruvate-3-^{14}C was used, the incubation medium contained 0.05 mc/ml (3.12 mc/m mole). Radioactive CO_2 was collected by absorption on hy-

* This investigation was supported by grant GY-517 from National Science Foundation.

† Present address: Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana.

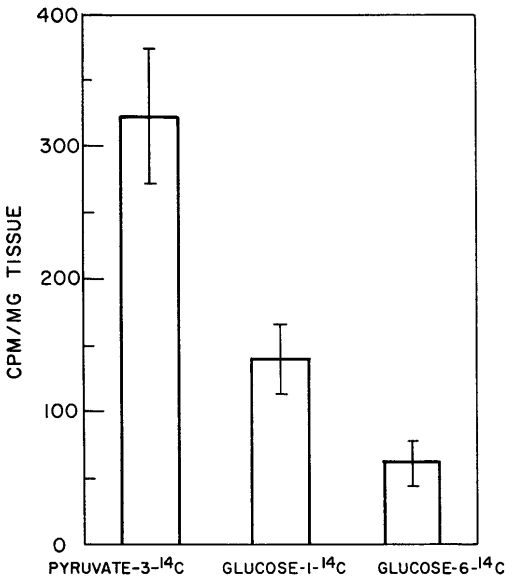

FIG. 1. Uterine $^{14}CO_2$ production from various substrates. Amounts of $^{14}CO_2$ produced during 1 hr incubation of whole, immature rat uteri weighing approximateely 30 mg. Incubation conditions are as described in *Methods*. Values are means ± SE based on six determinations from 2 experiments.

droxide of hyamine soaked filter paper in a center well of the flask(5). The filter paper was removed, placed in a scintillation vial with 10 ml of toluenephosphor (PPO-POPOP), and counted in a liquid scintillation counter. (^{14}C counting efficiency ~ 60%.) Lipid fractions were extracted from the uteri after incubation by methods previously described by Gorski and Nicolette(6).

Results. Uterine $^{14}CO_2$ production. Data on radioactive CO_2 produced from various labelled substrates by immature rat uterus are presented in Fig. 1. The high value observed with pyruvate suggests that pyruvate production may be rate-limiting or that pyruvate transport is greater than glucose transport in this tissue. The observation that $^{14}CO_2$ production from glucose-1-^{14}C is about twice that from glucose-6-^{14}C is probably due to the position of the labelled carbon in these two compounds. Glucose-1-^{14}C will contribute $^{14}CO_2$ when metabolized either by the Embden-Meyerhoff pathway or by the pentose phosphate cycle.

Lipid synthesis. Fig. 2 shows that the incorporation of radioactivity from various labelled substrates into uterine lipid is markedly increased 2 hours after *in vivo* estrogen treatment. Statistical analysis was made by a t-test of the difference between two sample means(7). The lipid extraction procedure used here is quite non-specific but other workers have shown that all classes of lipid are involved in this response(8). The effect of estrogen is nearly equal in the case of glucose and pyruvate.

Estrogen effects on $^{14}CO_2$. The data presented in Fig. 3 demonstrate that estrogen increases uterine $^{14}CO_2$ production with glucose as substrate but not with pyruvate as substrate. This effect is observed *in vitro* after previous hormone treatment *in vivo*. The rapidity of this response and the previous lipid synthesis response (within 2 hours) serves to identify these effects among the early uterine responses to estrogen. The small decrease of $^{14}CO_2$ from labelled pyruvate as compared with control values is a consistent finding although it is not statistically significant.

Discussion. Since estrogen effects on rat uterus occur shortly after hormone treatment, the delayed metabolic effects recently reported by Barker and Warren(9) probably

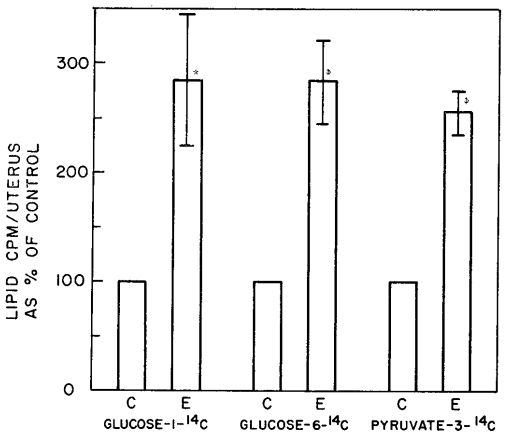

FIG. 2. Effect of estradiol on incorporation of labelled glucose and pyruvate into uterine lipid. Animals were injected 2 hr prior to killing with 5 μg estradiol-17β. Uteri were incubated for 1 hr. Experimental procedure as described in *Methods*. C = Control. E = estradiol treated. Values are means ± SE from 4 experiments with significance of the difference between estradiol treated animals and controls (t-test) indicated as follows: * = $p < .05$, Ø = $p < .01$. Typical control CPM/uterus are 262, 165, and 200 for glucose-1-^{14}C, glucose-6-^{14}C, and pyruvate 3-^{14}C, respectively.

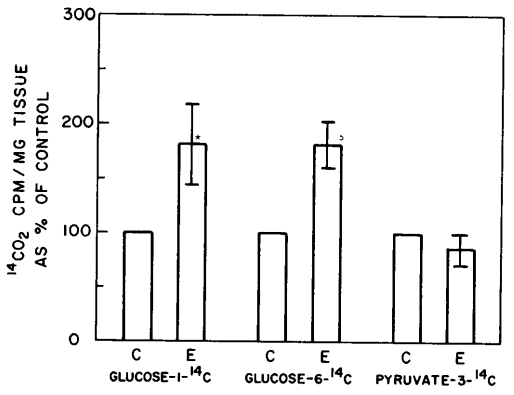

FIG. 3. Effect of estradiol on uterine $^{14}CO_2$ production from labelled glucose and pyruvate. Details of treatment and incubation same as for Fig. 2. Experimental procedures are as described in *Methods*. C = control. E = estradiol treated. Typical control CPM/mg values are as in Fig. 1. Values are means ± SE from 4 experiments and symbols for statistical significance are the same as for Fig. 2.

include events occurring during the amplification phase of the hormone response. Parameters which can be defined within the first 1 or 2 hours after treatment are more likely to be identified with the primary response(10). Nicolette and Gorski(4) have shown that estrogen causes the rapid appearance of radioactivity from labelled glucose in a number of end points of glucose metabolism. This finding led these authors to postulate that estrogen influences some early step in the metabolic sequence.

The observation that $^{14}CO_2$ output from glucose-1-^{14}C is approximately twice that from glucose-6-^{14}C (Fig. 1) shows that the Embden-Meyerhoff and pentose phosphate pathways contribute about equally to $^{14}CO_2$ production(11). The high levels of $^{14}CO_2$ produced with pyruvate-3-^{14}C as substrate occur despite the presence of 90 mg% glucose present in the Eagle's incubation medium.

Equal increases in incorporation into lipid of radioactivity from glucose-1-^{14}C and glucose-6-^{14}C (Fig. 2) are as expected since the tissue should not make any distinction between these two compounds in lipid synthesis. The slightly lower increase in incorporation of label from pyruvate as compared to glucose is not statistically significant, but it is consistent. It could be explained by the additional entry of glucose label into lipid *via* glycerol. Similar per cent increases produced by estrogen stimulation of glucose and pyruvate label into lipid would therefore locate the effect of estrogen beyond pyruvate in lipid synthesis.

The differential effect of estrogen on $^{14}CO_2$ production from labelled glucose and pyruvate (Fig. 3) indicates an acceleration of glucose metabolism in a reaction between these two substrates. Acceleration of metabolism beyond pyruvate would be reflected in an increase in $^{14}CO_2$ from both pyruvate and glucose. Also, acceleration beyond glucose-6-phosphate but prior to pyruvate would be reflected in a differential stimulation of $^{14}CO_2$ production from glucose-6-^{14}C and glucose-1-^{14}C. This would also be true for a site of acceleration in the pentose phosphate cycle. The similar per cent increase of $^{14}CO_2$ from these two glucose substrates illustrated in Fig. 3 indicates that the acceleration due to estrogen is prior to the branching of the Embden-Meyerhoff and pentose phosphate pathways. An alternative possibility is that the effect is due to responses of similar magnitude in both pathways. It is more probable, however, that estrogen is influencing either transport of glucose, phosphorylation of glucose, or both of these early steps in glucose metabolism.

The mechanism by which estrogen influences uterine glucose metabolism remains speculative at present. A general assumption is that a hormone's primary effect is on some rate-limiting step in a sequence of reactions. From the data presented here, one might predict that uterine hexokinase activity should increase within 2 hours after estrogen treatment. However, measurements by this author show that activity of this enzyme is not significantly increased until about 8 hours after hormone administration. This places this enzyme in the same category as uterine glucose-6-phosphate dehydrogenase (9) and *de novo* synthesized uterine phosphofructokinase recently reported by Singhal and Valadares(12). These *in vitro* enzyme assays have not further defined the early changes in uterine glucose metabolism. Generalized enzyme synthesis occurring beyond 6-8 hours probably account for the changes in enzyme

activities but occurs too late to explain the early metabolic events mentioned in this and previous papers(1,2,4).

Summary. Measurements of incorporation of ^{14}C from differentially labelled glucose and pyruvate into uterine lipid and CO_2 were used to evaluate early metabolic changes produced by estradiol in immature rat uterus. Tissue was incubated with the isotopes *in vitro* after hormone treatment *in vivo*. With pyruvate-3-^{14}C as substrate, substantially more $^{14}CO_2$ is produced than with glucose-1-^{14}C or glucose-6-^{14}C as substrates. Also, $^{14}CO_2$ output from glucose-1-^{14}C is about double that from glucose-6-^{14}C. Estradiol treatment causes a similar increase of ^{14}C incorporation into lipid with either of the glucose substrates or with pyruvate. This suggests that the estradiol effect on lipid synthesis is beyond pyruvate. A differential effect on $^{14}CO_2$ production from glucose and pyruvate, however, locates an estradiol effect between these substrates. Furthermore, the estradiol acceleration of $^{14}CO_2$ production from glucose-1-^{14}C and glucose-6-^{14}C is equal. This suggests that the site of estrogen stimulation of uterine glucose metabolism is prior to the formation of glucose-6-phosphate and therefore implicates the transport step, the phosphorylation step, or both.

The technical assistance of Mrs. Joan Hoffmann and the advice and suggestions of Dr. Jack Gorski are gratefully acknowledged.

1. Szego, C., Roberts, S., Recent Progr. Hormone Res., 1953, v8, 419.
2. MacLeod, R. M., Hollander, V. P., Fed. Proc., 1961, v20, 199.
3. Bitman, J., Cecil, H. C., Mench, M. L., Wren, T. R., Endocrinology, 1965, v76, 65.
4. Nicolette, J. A., Gorski, J., Arch. Biochem. Biophys., 1964, v107, 279.
5. Cuppy, D., Crevasse, L., Anal. Biochem., 1963, v5, 462.
6. Gorski, J., Nicolette, J. A., Arch. Biochem. Biophys., 1963, v103, 418.
7. Steel, R. G., Torrie, J. H., Principles and Procedures of Statistics, McGraw-Hill Book Co., New York, 1960, p72.
8. Aizawa, Y., Mueller, G. C., J. Biol. Chem., 1961, v236, 381.
9. Barker, K. L., Warren, J. C., Endocrinology, 1966, v78, 1205.
10. Gorski, J., Noteboom, W. D., Nicolette, J. A., J. Cell Comp. Physiol., 1965, v66, 91.
11. Wood, H. G., Katz, J., J. Biol. Chem., 1958, v233, 1279.
12. Singhal, R. L., Valadares, J. R. E., Life Sci., 1966, v5, 1299.

Received September 30, 1966. P.S.E.B.M., 1967, v124.

Effect of Guar Gum and Pectin N. F. on Serum and Liver Lipids Of Cholesterol Fed Rats. (31844)

B. A. RICCARDI AND M. J. FAHRENBACH (Introduced by B. L. Hutchings)
Biochemistry Research Department, Biochemical Research Section, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

Previous studies in our laboratory(1) have demonstrated the hypocholesterolemic effect of 16 mucilaginous polysaccharides in White Leghorn cockerels fed a semi-purified diet supplemented with dietary cholesterol. Because our results on the relative order of activity of the various mucilaginous polysaccharides in the chick did not agree with those of Ershoff and Wells in the rat(2), it appeared of interest to carry out several experiments in rats comparing guar gum and pectin N. F. under similar conditions.

Methods. Two basal diets were employed. The first of these was a casein-sucrose basal diet similar to that employed by Ershoff and Wells(2); the composition of this diet has been previously reported(3). The second diet was powdered Purina Lab Chow. In the casein-sucrose basal diet, test substances (including 1% cholesterol and 10% corn oil) were added at the expense of carbohydrate; in the Purina Lab Chow basal diet, test substances (including 1% cholesterol and 5% corn oil) were added at the expense of the