The Effects of Metyrapone and ACTH on the Development of Gluconeogenesis in the Neonatal Pig[1,2] (37643)

R. J. MARTIN, J. A. PATT, AND R. J. EBERHART
(Introduced by E. W. Hartsook)

Departments of Animal and Veterinary Science, The Pennsylvania State University, University Park, Pennsylvania 16802

The development of gluconeogenic capacity occurs very rapidly after birth in the rat (1), guinea pig (2), and neonatal pig (3, 4). The mechanisms by which these changes occur are probably mediated through changes in either hormone levels or tissue hormone sensitivities. Of the hormones known to regulate glucose homeostasis, the adrenal cortical hormones appear to be prominent in the stimulation of the gluconeogenic process. This is evident from the observations that adrenalectomy reduces or abolishes the gluconeogenic response to fasting, diabetes, or glucogon (5, 6) and that administration of adrenal cortical hormones causes a premature increase in glycogen storage in fetal rats (7).

Metyrapone has been shown to inhibit cortisol production in the pig by inhibition of 11β-hydroxylase (8). If cortisol is responsible for the rapid increase in the levels of gluconeogenic enzymes in the neonatal pig, then metryapone injections should prevent this development. The objectives of this study were to determine the effects of cortisol status of the neonatal pig on the early increases in liver and kidney gluconeogenic capacity.

Methods and Materials. Animals. Eleven pregnant Yorkshire sows with known breeding dates were Caesarean delivered on the 111th or 112th day after conception. The zero time was approximately 30 min after the first piglet had been removed from the sow. Each piglet was weighed and injected im with 1.0 ml/kg body weight of either 1 U/ml ACTH, 5 mg/ml metyrapone, or 44 m*M* sodium tartrate and 88 m*M* sodium chloride. All solutions were passed through a 0.45 μm millipore filter before use and injections were repeated 4, 8, and 12 hr after the initial injection. The piglets were fed bovine colostrum by stomach tube at 2, 10, 18, 26, and 34 hr after first injections. Piglets from each group were sacrificed at 0 (untreated), 6, 22, 30, and 38 hr. Blood was collected at the time of sacrifice after the pigs were stunned by a blow on the head. The livers and kidneys were quickly removed, weighed, and placed in cold saline. Kidney cortex slices (100 mg wet wt) were prepared with a Stadie-Riggs tissue slicer (0.5 mm thick) and incubated in 4 ml of Krebs Ringer bicarbonate buffer under a 95% O_2:5% CO_2 atmosphere for 1 hr at 38° (9). Substrates used to measure glucose production were 5 m*M* pyruvate and 5 m*M* glycerol. Glucose concentration in the media was determined by the glucose oxidase method (Sigma No. 970A). To determine the net glucose production from the substrate added, the blank flasks were subtracted from the flasks with substrate. The glucose values of blank flasks were usually 5–10% of the flasks containing substrate indicating very little endogenous release of glucose into the media.

The liver and kidney homogenates used for assay of glucose-6-phosphatase (G-6-Pase) were prepared in 0.1 *M* citrate buffer, pH 6.5. The assay of G-6-Pase (EC 3.1.3.9.) was performed on liver homogenates by measuring the release of phosphate from glucose-6-phosphate as described by Freedland and

[1] Partially supported by the Biomedical Sciences Support Grant RR-07082-06 from the General Research Support Branch, Division of Research Resources, Bureau of Health Professions, Education and Man Power Training, NIH.

[2] Authorized for publication November 21, 1972 as paper no. 4338 in the journal series of the Agricultural Experiment Station.

TABLE I. Blood Glucose (mg/100 ml) Levels of Experimental Pigs.

Treatment group	Age (hr)				
	0	6	22	30	38
Control	65.6	66.6	68.4	78.0	76.4
Metyrapone	—	66.5	50.8[a]	56.2[a]	59.8[a]
ACTH	—	68.5	101.8[b]	73.4	72.2

[a] Significantly different from control levels ($p < 0.05$).

[b] Highly significantly different from control levels ($p < 0.01$).

Harper (10). Other assays were performed on liver and kidney tissue supernatants prepared by homogenization (1:5 w/v) in 0.25 *M* sucrose containing 1 m*M* dithiothreitol and centrifugation at 27,000*g* at 4° for 20 min. The following assays were determined on a Gilford 2400 recording spectrophotometer at 25°: fructose diphosphatase (EC 3.1.3.11) by the procedure of Taketa and Powell (11), serine dehydratase (EC 4.2.1.13) by the methods of Freedland (12), aspartate aminotransferase (EC 2.6.1.10) by the method of Herzfeld and Greengard (13), and alanine aminotransferase (EC 2.6.1.2) by the procedure of Segal and Matsuzawa (14). All enzyme assays were linear with time and enzyme concentration. Enzyme activity was expressed as μmoles of product formed per minute per kg body weight. The procedure for determination of liver glycogen was essentially that described by Siu *et al.* (15).

Results. Blood glucose levels of the experimental pigs are presented in Table I. Metyrapone injections significantly lowered blood glucose levels of the fed neonatal pig at the ages 22, 30, and 38 hr. ACTH injections caused an elevation in blood glucose levels in pigs sacrificed at 22 hr. In general, blood glucose levels increased with increasing age. Liver glycogen levels were lowest in the metyrapone treated pigs and highest in the ACTH group (Table II).

The levels of liver G-6-Pase increased approximately 5-fold from zero age to 38 hr of age (Table III). ACTH did not influence this developmental pattern whereas metyrapone caused a decrease in magnitude of this developmental response at 22 and 30 hr but not at 38 hr of age. The same type of response was observed in kidney G-6-Pase levels, however, the magnitude of change from birth to 38 hr in the control group was only 2-fold (Table IV).

The level of fructose diphosphatase and alanine aminotransferase in both liver and kidney did not change with age or with treatment (Tables III and IV). Liver aspartate aminotransferase increased 2-fold by 30 hr of age. Metyrapone injections caused a decrease in this response at 30 hr of age (Table III).

Serine dehydratase which was not detected in kidney tissue samples showed the greatest developmental response in liver tissue (Table III). A 25-fold increase in this enzyme was observed between birth and 30 hr of age. During this time metyrapone injections significantly retarded the development of this enzyme. ACTH caused an increase in this enzyme at 22 and 38 hr when compared to the control (Table III).

Glucose production from pyruvate in kidney cortex slices is increased 2-fold from birth to 38 hr of age (Fig. 1). Metyrapone-injected pigs showed a delayed developmental response when compared to control and ACTH groups.

Glucose production from glycerol was greater than that from pyruvate (Fig. 2). In general, glucose production from glycerol in kidney cortex slices was enhanced by ACTH and depressed by metyrapone.

Discussion. During the first 24 hr postpartum the neonatal pig experiences a critical period of carbohydrate metabolism regulation. During this period the liver glycogen stores

TABLE II. Liver Glycogen Levels (mg/g tissue) of Experimental Animals.

Treatment group	Age (hr)				
	0	6	22	30	38
Control	120.1	50.1	15.4	8.2	15.1
Metyrapone	—	48.2	18.6	3.6[a]	2.3[b]
ACTH	—	61.2	27.9[a]	15.9[b]	18.9

[a] Significantly different from control levels ($p < 0.05$).

[b] Highly significantly different from control levels ($p < 0.01$).

TABLE III. Effects of Metyrapone and ACTH on Neonatal Pig Liver Enzymes.

Treatment	Age (hr)	Liver weight[a]	Liver tissue enzymes: Glucose-6-phosphatase	Fructose-1,6-diphosphatase	Serine dehydratase	Alanine aminotransferase	Aspartate aminotransferase
			μmoles/min/kg body weight				
Control	0	28.3 ± 1.4[b]	185 ± 49	57 ± 3	0.5 ± 0.1	118 ± 27	456 ± 64
Control	6	26.8 ± 1.2	292 ± 45	61 ± 3	0.2 ± 0.1	142 ± 29	471 ± 97
Metyrapone	6	26.7 ± 1.4	237 ± 43	68 ± 4	0.5 ± 0.1	132 ± 23	472 ± 44
ACTH	6	24.8 ± 1.0	260 ± 24	62 ± 2	0.3 ± 0.1	140 ± 10	450 ± 36
Control	22	21.5 ± 0.9	578 ± 49	50 ± 9	1.6 ± 0.4	158 ± 25	722 ± 83
Metyrapone	22	19.6 ± 0.8[c]	392 ± 22[d]	43 ± 4	0.4 ± 0.2[d]	165 ± 16	630 ± 81
ACTH	22	24.0 ± 0.9[c]	678 ± 81	59 ± 6	6.7 ± 1.8[d]	160 ± 17	881 ± 71
Control	30	20.9 ± 1.0	862 ± 115	51 ± 8	12.9 ± 2.6	124 ± 9	919 ± 79
Metyrapone	30	18.7 ± 1.1[c]	425 ± 65[c]	42 ± 6	0.2 ± 0.1[d]	124 ± 11	608 ± 41[d]
ACTH	30	23.4 ± 1.2[c]	834 ± 158	76 ± 9	6.4 ± 1.8[c]	157 ± 23	834 ± 92
Control	38	22.4 ± 0.9	906 ± 147	66 ± 8	6.2 ± 1.5	137 ± 8	814 ±119
Metyrapone	38	19.0 ± 1.1[c]	623 ± 52	44 ±12	1.0 ± 0.5[d]	120 ± 12	651 ± 38
ACTH	38	21.7 ± 1.1	965 ± 115	60 ± 4	11.4 ± 2.1[c]	155 ± 17	1053 ±152

[a] Liver weight is expressed as g per kg body weight.
[b] Mean ± SE for six animals.
[c] Significantly different from control levels ($p < 0.05$).
[d] Highly significantly different from control levels ($p < 0.01$).

which were deposited during the later stages of gestation are rapidly depleted, and the ability of the pig to withstand the stress of fasting is diminished (16). It has been postulated that the ability to maintain normal plasma glucose levels increases as the capacity for gluconeogenesis develops (3). This is evident from the observations of Mersmann (3) and Swiatek (17) who showed that in the neonatal pig there is a 3- to 5-fold increase in the gluconeogenic enzymes and a 2- to 3-fold increase in glucose synthesis in liver slices. The four key enzymes in gluconeogenesis (G-6-Pase, fructose diphosphatase, pyruvate carboxylase, and PEP carboxykinase) reach a maximum level in the pig liver by 2–3 days of age and decrease with age (3). The same enzymes in the neonatal rat liver reach maximum level between 5–10 days of age (1). The present report supports these findings and also demonstrates a rapid increase in liver serine dehydratase and aspartate aminotransferase but no significant change in alanine aminotransferase activity during the first 38 hr postnatally. Similar observations on alanine aminotransferase were made by Snell and Walker (18) in the neonatal rat. The kidney cortex was shown to possess a pattern of enzyme development that was similar, but not as dramatic as that of liver tissue. Other workers have demonstrated similar changes in enzyme pattern in the rat (19, 20), guinea pig (21), and sheep (22).

Glucose production from both glycerol and pyruvate increased 2- to 3-fold during the early neonatal period. Gluconeogenesis from glycerol bypasses two key enzymatic steps (PEP carboxykinase and pyruvate carboxylase) that are required for gluconeogenesis from pyruvate. Since glycerol was utilized more rapidly than pyruvate for glucose production it would appear that these two enzymatic steps prior to α-glycerol-PO_4 formation were limiting pyruvate incorporation to glucose. It has been proposed that in the neonatal rat the appearance of PEP carboxykinase is critically important to the development of gluconeogenesis (23). Our observations in the pig kidney cortex support this theory.

Very little has been done to elucidate the hormonal requirements for the development of gluconeogenesis in the neonate. Studies

TABLE IV. Effects of Metyrapone and ACTH on Neonatal Pig Kidney Cortex Enzymes.

Treatment	Age (hr)	Kidney weight[a]	Kidney cortex tissue enzymes: Glucose-6-phosphatase	Fructose-1,6-diphosphatase	Alanine aminotransferase	Aspartate aminotransferase
			μmoles/min/kg body weight			
Control	0	6.3 ± 0.2[b]	38.6 ± 4.2	18.5 ± 1.2	3.1 ± 0.5	67.3 ± 9.1
Control	6	6.6 ± 0.4	49.9 ± 7.0	16.9 ± 1.7	4.4 ± 0.5	68.5 ± 3.1
Metyrapone	6	6.7 ± 0.1	46.3 ± 3.9	23.2 ± 2.4	3.6 ± 0.1	74.4 ± 6.0
ACTH	6	6.0 ± 0.2	38.6 ± 4.6	16.9 ± 0.5	2.9 ± 0.2	55.6 ± 3.5
Control	22	6.5 ± 0.1	89.0 ± 7.1	17.0 ± 1.5	3.7 ± 0.6	63.1 ±10.6
Metyrapone	22	6.4 ± 0.1	64.3 ± 8.1[c]	20.4 ± 1.9	3.6 ± 0.2	69.6 ± 3.3
ACTH	22	6.5 ± 0.2	86.7 ± 8.3	20.2 ± 1.7	3.7 ± 0.2	78.2 ± 5.2
Control	30	6.9 ± 0.1	83.0 ± 7.3	20.2 ± 1.3	3.8 ± 0.5	72.9 ± 8.6
Metyrapone	30	6.4 ± 0.3	53.4 ± 7.4[c]	20.6 ± 2.1	3.4 ± 0.4	67.5 ± 8.0
ACTH	30	7.0 ± 0.1	78.4 ± 6.1	21.1 ± 1.2	3.1 ± 0.3	69.8 ± 5.4
Control	38	6.7 ± 0.2	87.1 ± 8.5	20.8 ± 0.9	3.0 ± 0.4	65.3 ± 3.5
Metyrapone	38	6.8 ± 0.2	66.3 ± 5.8[c]	20.5 ± 1.2	2.7 ± 0.2	64.3 ± 8.4
ACTH	38	6.8 ± 0.1	120.1 ±12.0[c]	19.8 ± 0.9	3.3 ± 0.5	76.4 ± 7.5

[a] Kidney weight is expressed as g per kg body weight.
[b] Mean ± SE for six animals.
[c] Significantly different from control levels ($p < 0.05$).

with the rat have shown that premature delivery causes a rapid increase in enzymes associated with gluconeogenesis (24). Adrenal cortical hormones have been implicated in this developmental process. Adrenalectomy at birth prevents the normal increase in rat liver urea synthesizing enzymes and administration of corticosterone permits the normal developmental pattern to be achieved (25). Glycogen deposition in fetal rat liver was produced prematurely by the injections of cortisol to intact fetuses *in utero* (7). In the present study glycogen mobilization was not altered by altering the cortisol status of the neonatal pig but ACTH treated pigs appeared to maintain a higher level of liver glycogen than the metyrapone treated group at 22–38 hr of age. Metyrapone did not prevent the development of G-6-Pase in this study. This may be due to the fact that G-6-Pase begins to increase before birth in the neonatal pig, indicating that the initiation of enzyme synthesis occurs before birth (3, 4). Both liver and kidney fructose diphosphatase and alanine aminotransferase showed very little developmental change during the first 38 hr of age. Liver aspartate aminotransferase did increase 2-fold from birth to 30 hr of age and metyrapone prevented maximal development of this enzyme. Herzfeld and Green-

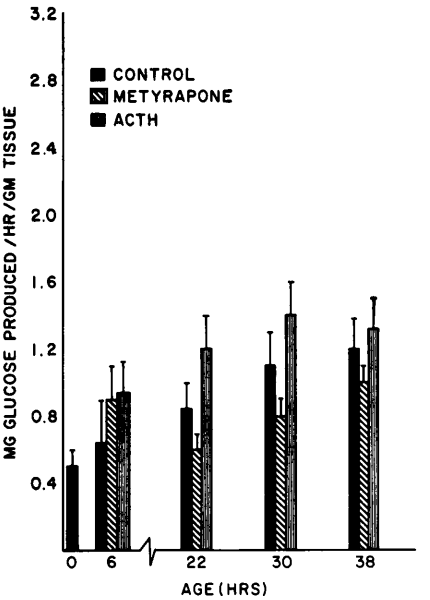

FIG. 1. The effect of metyrapone and ACTH on the conversion of pyruvate to glucose by neonatal pig kidney slices. The bars represent the mean ± SE of six animals.

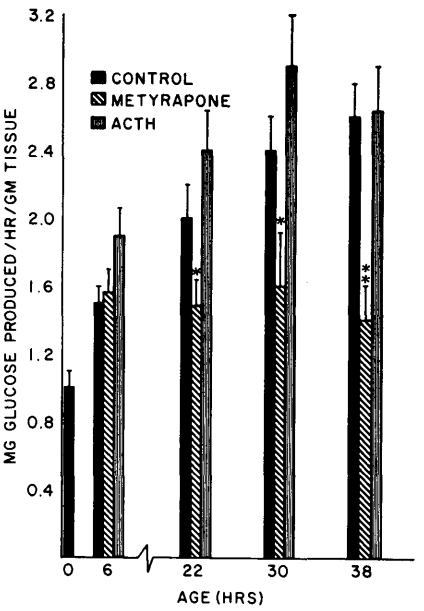

FIG. 2. The effect of metyrapone and ACTH on the conversion of glycerol to glucose by neonatal pig kidney slices. The bars represent the mean ± SE of six animals. * Significantly different from control values ($p < 0.05$). ** Highly significantly different from control values ($p < 0.01$).

gard (13) found a stimulatory effect of glycocorticoids on this enzyme in the neonatal rat liver. The lack of alanine aminotransferase response to cortisol status in this study is in agreement with experiments by Nichol and Rosen (26), which demonstrated that adrenalectomy during the neonatal period had no effect on the level of alanine aminotransferase in rat liver.

The greatest response to cortisol status was the liver serine dehydratase level. Metyrapone prevented the increase of this enzyme in the neonatal pig liver. Injection of hydrocortisone into rat fetuses is without effect on the prenatal development of this enzyme (27). However, a single injection of glucagon will cause a premature increase in serine dehydratase (27) as well as threonine dehyratase (28) and tyrosine aminotransaminase (29). The differences in developmental responses to these hormones may be caused by the fact that both hormones may be required for enzyme induction (30) and the differences in endogenous secretions of both hormones pre and postnatally (31, 32). It has also been proposed that liver enzymes develop in clusters and these clusters develop at different stages of growth (33).

In addition to glucagon and cortisol, other hormones have been shown to influence the development of gluconeogenic enzymes. Administration of either insulin or adrenalin to pregnant rats evokes a rapid increase in fetal liver threonine dehydratase (34). Adrenalin injection will also stimulate the development of PEP carboxykinase (35). It is thought that both of these hormones act by lowering blood glucose, thereby inducing glucagon release from the fetal pancreas. Growth hormone injections appear to have no influence on the early development of liver gluconeogenic enzyme (28). Thyroxine injections to either fetal or neonatal rats cause a premature rise in G-6-Pase and fructose diphosphatase (36). The current study permits only the evaluation of the effects of cortisol status on those enzymes which develop during the early neonatal stage. More work is required to determine the hormonal requirements for fetal and neonatal development of gluconeogenesis.

Summary. Pigs taken by Caesarean delivery on either 111th or 112th day of gestation were assigned to three treatment groups, each receiving im injections. The control group (C) received saline injections; the cortisol-deficient group (M) received 5 mg/kg body weight of metyrapone, and the ACTH group (A) received 1 U/kg body weight of ACTH. Pigs in group M demonstrated hypoglycemia at ages 22, 30, and 38 hr. The metyrapone-treated pigs had the lowest liver glycogen levels at ages 30 and 38 hr. Liver glucose-6-phosphatase activity increased 5-fold by 30 hr of age in groups C and A, and about 3-fold in group M. The development of liver serine dehydratase was dependent on an adequate level of plasma cortisol. Liver and kidney fructose-1,6-diphosphatose, alanine aminotransferase, and aspartate aminotransferase were not drastically altered by the cortisol status of the neonatal pig. Gluconeogenesis from pyruvate and glycerol in kidney cortex slices from pigs in group M was consistently lower than both groups A and C

pigs. These studies indicate that the rapid development of liver gluconeogenic enzymes in young pigs after birth is not dependent on high levels of cortisol during the period of development. However, maximum development of gluconeogenic capacity was prevented by cortisol deficiency.

The authors wish to acknowledge the excellent technical assistance of Mrs. Patricia Lamprey.

1. Yeung, D., Stanley, R. S., and Oliver, I. T., Biochem. J. **105**, 1219 (1967).

2. Kornfeld, R., and Brown, D. H., J. Biol. Chem. **238**, 1604 (1963).

3. Mersmann, H. J., Amer. J. Physiol. **220**, 1297 (1971).

4. Swiatek, K. R., Biochem. Biophys. Acta **252**, 274 (1971).

5. Friedmann, N., Exton, J. H., and Park, C. R., Fed. Proc. **27**, 625 (1968).

6. Exton, J. H., Mallette, L. E., Jefferson, L. S., Wong, E. H., Friedmann, N., Miller, T. B., and Park, C. R., Recent Prog. Horm. Res. **26**, 411 (1970).

7. Greengard, O., and Dewey, H. K., Develop. Biol. **21**, 452 (1970).

8. Stith, R. D., and Bottoms, G. D., Amer. J. Vet. Res. **33**, 963 (1972).

9. Krebs, H. A., Bennett, D. A., deGasquet, P., Gasocyne, T., and Yoshida, T., Biochem. J. **86**, 22 (1963).

10. Freedland, R. A., and Harper, A. E., J. Biol. Chem. **228**, 743 (1957).

11. Taketa, K., and Powell, B. M., Biochem. Biophys. Res. Comm. **12**, 229 (1963).

12. Freedland, R. A., J. Biol. Chem. **239**, 3357 (1964).

13. Herzfeld, A., and Greengard, O., Biochem. Biophys. Acta **237**, 88 (1971).

14. Segal, H. L., and Matsuzawa, T., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol 17, p. 153. Academic Press, New York (1962).

15. Siu, L., Russell, J. C., and Taylor, A. W., J. Appl. Physiol. **28**, 234 (1970).

16. Swiatek, K. R., Kipmis, D. M., Mason, G., Chao, K. L., and Cornblath, M., Amer. J. Physiol. **214**, 400 (1968).

17. Swiatek, K. R., Chao, K., Chao, H., Cornblath, M., and Tildon, J. T., Biochem. Biophys. Acta **222**, 145 (1970).

18. Snell, K., and Walker, D. G., Biochem. J. **128**, 403 (1972).

19. Burch, H. B., Oliver, L. H., Kuhlman, A. M., Skeyance, J., Diamant, E. J., Lowry, S. R., and Dippe, P., J. Biol. Chem. **238**, 2267 (1963).

20. Ballard, F. J., and Oliver, I. T., Biochem. J. **90**, 261 (1964).

21. Lea, M. A., and Walker, D. J., Biochem. J. **91**, 417 (1964).

22. Ballard, F. J., and Oliver, I. T., Biochem. J. **95**, 191 (1965).

23. Philippidis, H., and Ballard, F. J., Biochem. J. **113**, 651 (1969).

24. Dawkins, M. J. R., Brit. Med. Bull. **22**, 27 (1966).

25. Raiha, N. C., and Suihkonen, J., Biochem. J. **107**, 793 (1968).

26. Nichol, C. A., and Rosen, F., Adv. Enzyme Reg. **1**, 341 (1963).

27. Greengard, O., and Dewey, H. K., J. Biol. Chem. **242**, 2986 (1967).

28. Yeung, Y. G., and Yeung, D., Int. J. Biochem. **3**, 180 (1972).

29. Greengard, O., Biochem. J. **115**, 19 (1969).

30. Exton, J. H., Friedmann, N., Wong, E. H., Brineaux, J. P., Corbin, J. D., and Park, C. R., J. Biol. Chem. **247**, 3579 (1972).

31. Girard, J., Bal, D., and Assan, R., Hom. Metab. Res. **4**, 168 (1972).

32. Jackson, B. T., and Piasecki, G. J., Endocrinology **85**, 875 (1969).

33. Greengard, O., and Jamdar, S. C., Biochem. Biophys. Acta **237**, 476 (1971).

34. Yeung, Y. G., and Yeung, D., Comp. Biochem. Physiol. **44B**, 849 (1973).

35. Yeung, Y. G., and Oliver, I. T., Biochem. J. **108**, 325 (1968).

36. Greengard, O., and Dewey, H. K., J. Biol. Chem. **243**, 2745 (1968).

Received May 15, 1973. P.S.E.B.M., 1973, Vol. 144.