PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 162, 131–138 (1979)

Serum Growth Hormone, Somatomedin and Its Carrier Protein in the Rat: Influence of Age, Sex, and Pregnancy (40632)

BORIS DRAZNIN,[1] HELEN G. MORRIS, PHILIP J. BURSTEIN, AND DON S. SCHALCH

Department of Medicine, Division of Endocrinology, University of Colorado Medical Center, Denver, Colorado 80262

The physiology and hormonal regulation of growth is a highly complex process, many details of which remain to be elucidated. Although growth hormone (GH), somatomedins (SMs), and their carrier protein(s) (CPs) have been identified as major components of the growth-promoting system, little data are available concerning the interaction of these factors either on the growth of the placental–fetal unit or on that of organisms from fetal to adult life. Somatomedins comprise a group of four low-molecular-weight peptides (SM A, SM C, insulin-like growth factor [IGF], and multiplication stimulating activity), the biological activities of which are GH dependent (1, 2) and appear to be modulated by circulating CP concentrations (3–8). To gain further understanding about the growth-promoting system, we have examined the changes in serum levels of GH, IGF, and its CP in pregnant rats during normal gestation and in growing rats from fetal through adult life. Since prostaglandins (PGs) have been shown to act at both the hypothalamic and pituitary levels to stimulate GH release (9, 10), it seemed reasonable that their rise during gestation might contribute to the modulation of the growth-promoting system both in the maternal and fetal circulations. Therefore, the present study also reports the influence of the PG synthetase inhibitor, indomethacin, on serum levels of PGs, GH, IGF, and its CP in pregnant rats and their offspring.

Material and methods. Sprague–Dawley rats were housed in an air-conditioned room under controlled conditions of light (0600–2000) and temperature (22°). Purina laboratory chow and water were administered *ad libitum* to adult rats, while pups received mother's milk. Adult male (n = 18) and female (n = 7) rats were housed separately in groups of 4–5 animals per cage; pups (10–12/dam) stayed with their mothers in individual cages until weaned at Day 20. After weaning, pups were separated according to sex and 4–5 were housed per cage. All animals were weighed prior to each bleeding and blood samples drawn under light ether anesthesia from all except fetal and neonatal animals between 8 and 11 AM. Blood was obtained by decapitation from fetuses on the 20th day of gestation (n = 50), and from neonates (birth to 3 days) at 1 hr, 1 day, and 3 days after birth (n = 20, 18 and 20 pups, respectively). Blood samples from 4 to 5 fetuses, or neonates, were pooled for subsequent assays, while blood sampling was performed by tail artery catheterization approximately weekly (male), or monthly or bimonthly (female), from ages 14 through 120 days. Eleven pregnant rats were studied from the 4th to the 20th day of pregnancy; 6 were untreated while 5 received indomethacin (Merck, Sharpe, and Dohme) (10 mg/kg body wt i.m. twice a day) beginning on the 2nd gestational day. Blood samples were drawn from the tail artery on the 4th, 13th, and 20th day of gestation, and from the uterine vein immediately before the removal of the fetuses under light ether anesthesia on Day 20.

Blood samples obtained from all animals were centrifuged immediately and the sera kept frozen at −20° until assayed for GH by radioimmunoassay (RIA) (11), and IGF and its CP by competitive protein binding (CPB) assays (12, 13). While it is generally agreed that considerable biological, and probably structural, similarity exists between various SMs, rat SM appears to be a basic peptide most closely resembling SMs C and IGF I

[1] Please address requests for reprints to Dr. Boris Draznin, Department of Medicine, Endocrine Division, University of Colorado Medical Center, 4200 E. Ninth Avenue, Box B151, Denver, Colo. 80262.

0037-9727/79/090131-08 $01.00/0

(14). Moreover, it has been shown that SM MSA (multiplication stimulating activity), like SM C, cross-reacts both with IGF I and (to a lesser extent) II in the CPB assay (15, 16) and is detectable in rat plasma by RIA (17). While the CPB assay measures peptides which cross-react with both IGF I and II, it appears that the absolute rat SM levels, herein expressed as IGF reactivity, would be underestimated by this assay since it has been shown that CP has an affinity for IGF I which is approximately only 10% that for IGF II (18). Despite the known limitation in the specificity of the CPB assay, it is felt that it provides a useful approximation of the changes in rat SM levels which have been quantitated in terms of μU/ml relative to an IGF standard, the potency of which has been determined by its stimulation of glucose oxidation in the rat epidymal fat pad bioassay. Samples of maternal and fetal blood used for PG assays were immediately placed in tubes containing 2% EDTA and 10 μg aspirin to inhibit further PG synthesis by platelets *in vitro* during centrifugation. Specific RIAs were employed to measure the principal metabolite of PGF_{2_α} (13,14-dihydro-15-keto-PGF_{2_α}) (PGF M) in maternal plasma and PGE_2 in fetal plasma (19, 20). For statistical analysis, either the paired or unpair Student's *t* test was used as indicated.

Results. A. Growth and the growth-promoting system in growing rats. Consistent weight gain, from 5.0 ± 0.03 g (mean ± SEM) at birth to 424 ± 4 g at 120 days, was observed throughout the experiment. However, the slope of the curve for body weight varied at different ages (Fig. 1). Thus, the initial weight gain (3.6 g/day) between Days 3 and 28, reached its highest rate (6.0 g/day) between Days 28 and 55, then declined slightly (4.9 g/day) between Days 55 and 79, and was minimal (1.4 g/day) between Days 79 and 120. However, the fractional growth velocity in growing rats progressively declined from the birth on (data not shown) in compliance with natural process of growth. The pattern in weight gain was not significantly different in male and female animals during any of the time periods studied.

Despite the well-known broad range of GH concentrations in rats, the mean GH levels appeared to be age related (Fig. 1). Thus, the moderately high GH concentration in the fetus (137 ± 21 ng/ml) rose almost three-fold on the first day of life (371 ± 37 ng/ml, $P < 0.001$), decreased between Days 14 and 28, and reached a second peak at 48 days (365 ± 97 ng/ml) before decreasing to the adult level (30 ± 4 ng/ml) at 120 days.

During the first month of life the changes in serum IGF levels followed those in GH concentration ($r = 0.901$; $P < 0.001$). A mean fetal serum level of 812 ± 164 μU/ml rose significantly ($P < 0.001$) on the first day of life to 2108 ± 26 μU/ml. Thereafter, serum IGF rapidly declined to a nadir of 189 ± 26 μU/ml at Day 21, and then rose gradually to a level of 512 ± 12 μU/ml on Day 120 (Fig. 1).

The pattern of IGF CP concentration resembled that of weight gain rather than those of serum GH and IGF (Fig. 1). The CP levels in fetal (0.28 ± 0.01 mg/ml) and neonatal (0.36 ± 0.01 mg/ml, Day 3) circulations were very low, but doubled by Day 21. Thereafter, the mean CP level demonstrated a progressive rise until Day 41 (2.4 ± 0.2 mg/ml), and then remained at a plateau (range, 2.6 ± 0.11 to 2.9 ± 0.1 mg/ml) from 55 until 120 days. No significant differences were noted in serum GH, IGF, or CP levels between male and female rats at any age.

B. Growth-promoting system in pregnant rats. The body weights of controls (500 ± 9 g) and indomethacin-treated (528 ± 12 g) pregnant animals were virtually identical at term, as were those of their offspring (5.0 ± 0.1 and 5.1 ± 0.2 g, respectively). The level of PGF M in the systemic circulation of pregnant controls demonstrated a significant ($P < 0.01$) rise from the 4th (152 ± 22 pg/ml) to the 13th day (256 ± 49 pg/ml), and remained elevated on the 20th day of gestation (235 ± 29 pg/ml). The administration of indomethacin significantly ($P < 0.01$) reduced the levels of PGF M in systemic circulation of pregnant rats at all time periods studied (Fig. 2).

While serum GH levels were similar both in control and indomethacin-treated pregnant rats on the 4th and 13th days of gestation, by the 20th day the mean GH concentration was almost 50% less in the indomethacin-treated group than in the controls. Serum IGF levels both in the control and in-

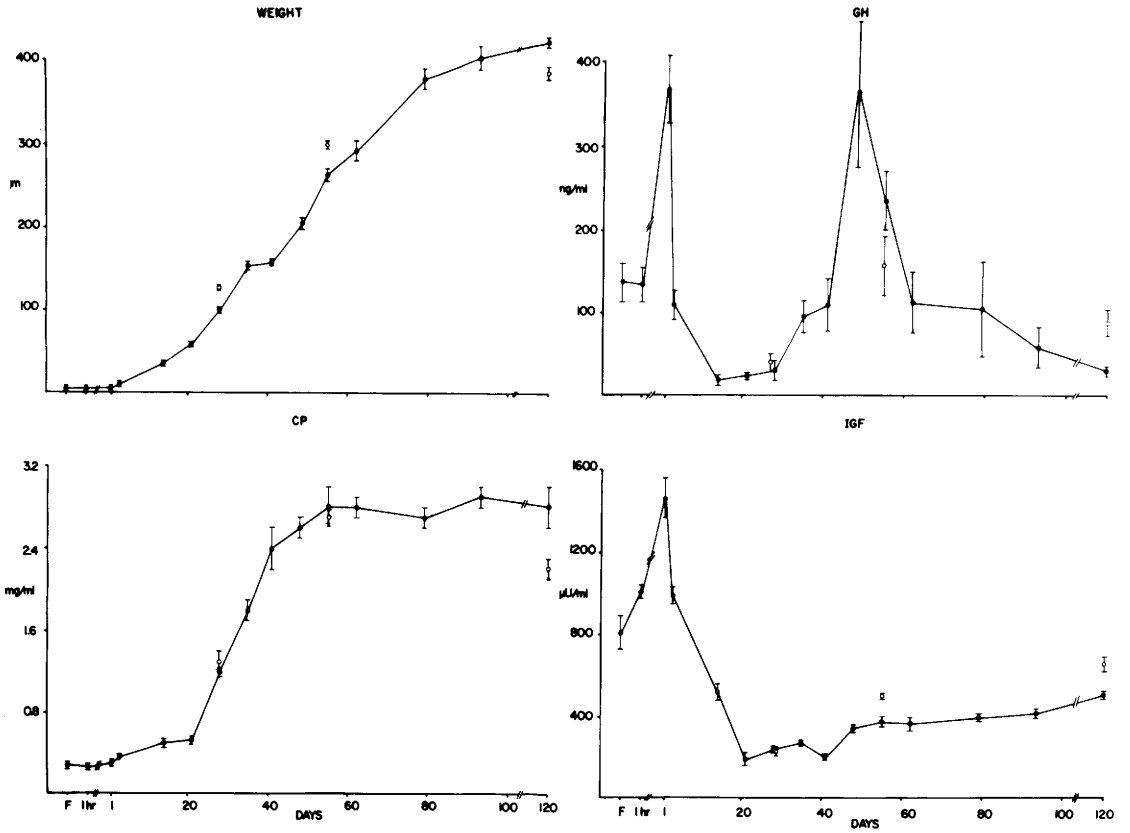

FIG. 1. Body weight and serum GH, IGF, and CP levels in rats. Results are expressed as mean ± SEM. Groups of fetal (F) and neonatal rats include the animals of both sexes (◒). At age 20 days male (●) and female (○) rats were segregated; no statistically significant sex-related differences in any component of the growth-promoting system were observed.

domethacin-treated rats exhibited a gradual decline throughout the gestational period, from 250 ± 11 to 128 ± 3 μU/ml in controls, and from 308 ± 6 to 193 ± 16 μU/ml in indomethacin-treated animals (4th and 20th day, respectively). In contrast to reduced GH concentrations, the mean IGF in indomethacin-treated rats was slightly but significantly ($P < 0.01$) higher than in control animals throughout gestation. A dramatic and highly significant ($P < 0.001$) drop in IGF CP concentration was observed in all pregnant animals, with and without indomethacin treatment. The slopes of the decrease in CP from Days 4 to 13 were steep and parallel, dropping from 2.54 ± 0.1 to 0.29 ± 0.11 mg/ml in controls, and from 2.43 ± 0.2 to 0.25 ± 0.11 mg/ml in indomethacin-treated rats.

In the control animals the PGF M concentration in the uterine vein on Day 20 (386 ± 54 pg/ml) was significantly ($P < 0.001$) higher than in the systemic circulation (Fig. 3). Indomethacin administration caused a striking reduction ($P < 0.005$) in PGF M concentration both in the systemic and uterine circulation (99 ± 5 and 60 ± 10 pg/ml, respectively) on the 20th gestational day. Similarly, the plasma PGE_2 level in the fetuses of indomethacin-treated mothers (398 ± 152 pg/ml) was significantly ($P < 0.01$) lower than in those of the control mothers (913 ± 42 pg/ml). The administration of indomethacin to pregnant rats caused a significant decrease not only in their plasma PG concentrations, but in serum GH levels as well. The latter were reduced both in the systemic circulation (89 ± 5 vs 50 ± 5 ng/ml, $P < 0.01$) and in the uterine vein (89 ± 8 vs 59 ± 5 ng, $P < 0.01$). The fetal GH concentration in the indomethacin-treated rats (83

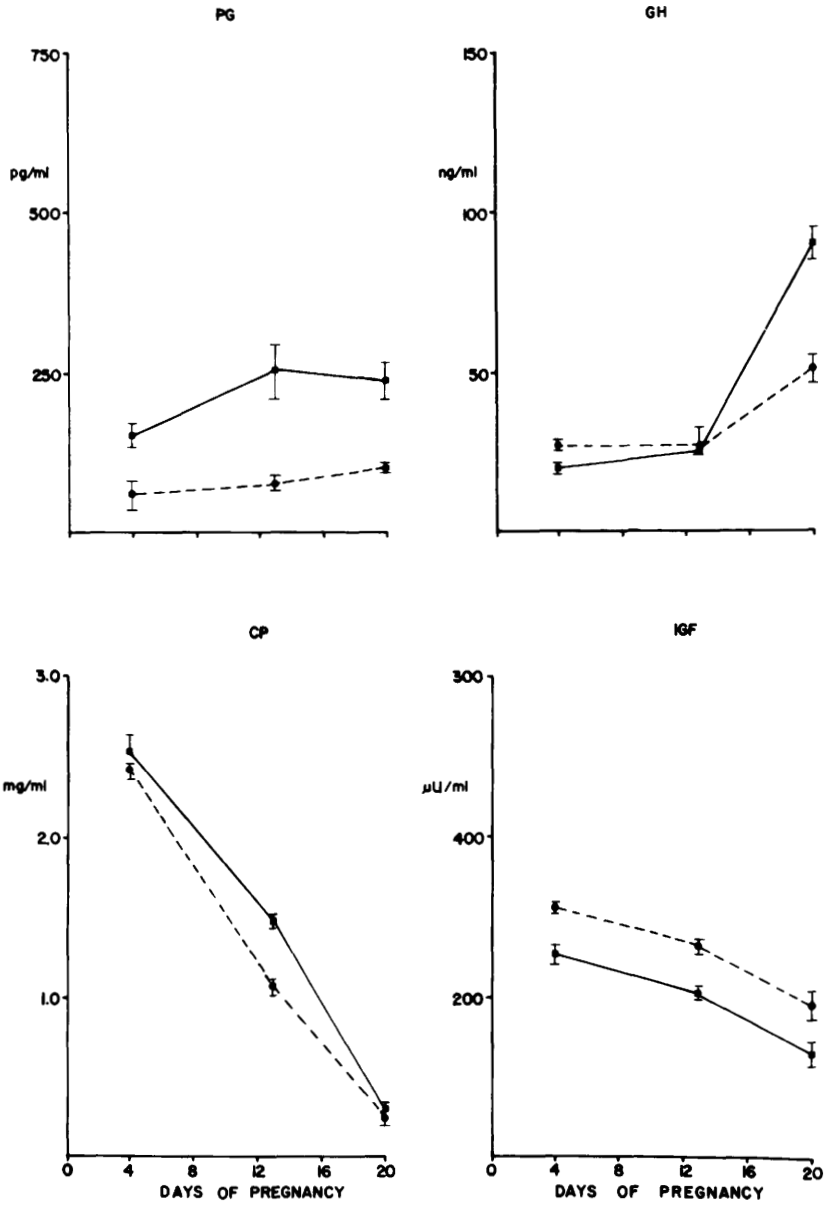

FIG. 2. Plasma PG (PGF M) and serum GH, IGF, and CP levels in control (■——■) and indomethacin-treated (●--●) pregnant rats. Indomethacin (10 mg/kg) was injected i.m. twice a day starting on the second gestational day. Results are expressed as mean ± SEM.

± 7 ng/ml) was also significantly lower ($P < 0.05$) than in fetuses of the control group (137 ± 21 ng/ml). The levels of IGF in both maternal systemic circulation and uterine vein of indomethacin-treated rats were significantly higher ($P < 0.001$) than those of control animals. The fetal IGF concentrations, however, were found to be similar both in the control group (812 ± 64 µU/ml) and the indomethacin-treated animals (726 ± 36 µU/ml). The mean CP concentrations in the fetal, maternal systemic circulation, and in the uterine vein were virtually identical in both groups.

Discussion. The regulation of growth is dependent upon a highly complex system which

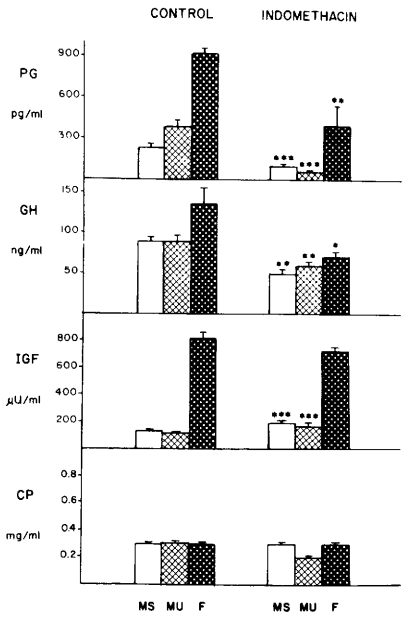

FIG. 3. Plasma PGF M (maternal) and PGE_2 (fetal) and serum GH, IGF, and CP levels in control and indomethacin-treated pregnant rats and their offspring on the 20th gestational day. Values given for the maternal systemic circulation (MS), maternal uterine vein (MU), and fetal circulation (F) are expressed as mean ± SEM. *P* values vs control: * <0.05, ** <0.01, and *** <0.001.

involves the interaction of GH, SMs, and SM carrier proteins. Naturally, GH has a central role in this process by inducing the synthesis and release of SMs which, in turn, mediate many of the effects of GH on peripheral cells (1, 2, 21, 22). Moreover, the biological activity of the SMs appears to be regulated in part by circulating levels of their CPs (3–8, 23, 24). Although many of the components of the growth-promoting system have been identified, the factors which govern the release and action of the interacting substances remain unclear. In this study, we have examined the influence of several factors on the growth-promoting system in the rat. This animal was selected for our studies because in contrast to the human, the rat continues to grow throughout life.

There are two periods during the life of young rats in which serum GH levels appear to be closely related to the rate of growth. The first is during the late fetal and early neonatal life when serum GH is markedly elevated, a finding long recognized both in humans (26) and in rats (27). The importance of elevated GH levels in newborns is emphasized by the observation of marked growth retardation following hypophysectomy at this age (28). The present data, in agreement with these of Ojeda and Jameson (25), demonstrate that high postnatal GH concentrations coincide with a sevenfold increase (from 5 to 36 g) in body weight from birth to age 14 days. Factors regulating the pattern of GH secretion in early infancy are still largely unknown. Although a causative role of estrogens at this age has been suggested, it has also been shown that estradiol treatment does not alter GH synthesis in overiectomized rats (29). Walker *et al.* (30) have demonstrated a highly significant inverse correlation between the hypothalamic somatostatin and serum GH concentrations in neonatal rats, and have emphasized a physiological role in the postnatal development of the hypothalamic regulatory mechanism for pituitary GH release.

The second peak in serum GH concentration occurs prior to sexual maturation (25), coincident with an alteration in growth rate. Our data indicate that the rate of weight gain in male animals (initially 3.6 g/day) almost doubled from Days 28 to 55 (6.0 g/day). Although the rat does not exhibit as marked a "pubertal growth spurt" as the human, Ojeda and Jameson (25) have also found that plasma GH concentrations increase in female rats prior to puberty and are associated with an increase in the slope of the curve for body weight. The increase in GH concentrations during puberty may be related to the influence of the sex steroids which are known to stimulate the release of GH (34, 35).

In contrast to many reports of low SM activity in cord blood of human beings (36–38) and in neonatal rats (39), we have found high levels of the somatomedin IGF in the circulation of fetal and newborn rats. The initial changes in serum IGF follow the changes in GH concentration. As with GH, the elevated level of IGF rose further on the first postnatal day and then decreased to its lowest point by Day 21, following the nadir in serum GH. Thereafter, the IGF concentration demonstrated a continuous but gradual increase to Day 120, departing from the pattern of GH secretion which rose to a second peak at Day 48. Serum IGF CP demonstrated

an entirely different profile. Extremely low in early neonatal circulation, the level of IGF CP rose slowly by the 21st day, exhibited an accelerated rise between Days 21 and 41, and reached a plateau on the 55th day. Although there was no apparent correlation between IGF CP and either GH or IGF levels, there was a striking similarity between the changes in CP levels and the pattern of weight gain.

Pregnancy, with its multihormonal changes, induces new factors into the regulation of the growth-promoting system. Growth hormone secretion and the amplitude of individual pulses in pregnant rats have been shown to be increased on gestational Days 18–21 (40). We have also observed a significant rise in GH levels in control pregnant animals on the 20th day of gestation. Nevertheless, despite this obvious rise, the mean maternal GH concentration was far less than that of the offspring. A slight, but significant, decrease in IGF concentration throughout gestation and the unexpectedly high fetal IGF levels pose additional questions and emphasize the differences between the rat and human growth-promoting systems. Thus, in pregnant women, the mean IGF concentration exhibits a tendency to rise while its level in cord blood is barely half that in the maternal circulation; in contrast, the decrease of IGF CP level in pregnant rats was similar to that observed in humans (41). The physiological significance and possible cause(s) of this reduction in CP concentration during pregnancy are unknown. An identical observation that had been noted in the serum of pregnant women (41) led us to investigate the possibility of a rise in serum-"free" IGF concentration. The data obtained have demonstrated that "free" IGF levels in pregnant women were approximately threefold higher than those in nonpregnant controls (manuscript submitted for publication).

Since PGs have been repeatedly shown to increase GH release (31–33), a significant rise in their concentration during gestation may contribute to the elevation in maternal GH concentration in late pregnancy and to that in the fetal–neonatal circulations. PG measurements in plasma provide only an indirect index of synthesis by various tissues. Nevertheless, the significant decreases in PG concentration at three sites (maternal systemic, maternal uterine, and fetal circulations) attest to the widespread inhibition of PG synthesis induced by indomethacin; the blockage affects all compounds derived from the cyclooxygenase pathway. Lower concentrations of PGs in indomethacin-treated pregnant rats and their offspring were accompanied by lower GH levels as compared to controls. Surprisingly, the IGF levels in maternal circulation in indomethacin-treated animals were slightly, but significantly, higher than in the control group. Furthermore, in spite of substantial differences in GH concentration, IGF levels and fetal weights were similar in indomethacin-treated and control animals. This apparent lack of positive correlation between serum GH and IGF levels in indomethacin-treated rats suggests that substances other than GH may govern SM production in pregnant rats. Thus, Daughaday and his colleagues (42, 43) have recently demonstrated that in rats hypophysectomy performed on the 14th gestational day was not accompanied by decrease in SM concentration until after delivery. They have concluded, therefore, that SM concentration in pregnant rats was probably maintained by placental somatomammotropin.

Summary. The changes in body weight and serum levels of growth hormone (GH), somatomedin insulin-like growth factor (IGF), and its carrier protein (CP) have been followed in rats from fetal to adult life. The weight gain was maximal (6.0 g/day) between Days 28 and 55 and minimal (1.4 g/day) between Days 79 and 120. Two distinct peaks of GH concentration have been found in normally growing animals. The first (mean ± SEM) was observed on the first day of life (371 ± 37 ng/ml) while the second peak (365 ± 97 ng/ml) coincided with an acceleration in growth rate beginning Day 40. An unexpectedly high fetal IGF level (812 ± 164 μU/ml) rapidly declined to a nadir (189 ± 26 μU/ml) at Day 21, and then rose gradually to a level of 572 ± 12 μU/ml on Day 120. The pattern of IGF CP concentration resembled that of weight gain rather than those of serum GH and IGF. The sex of animal appeared to have no influence on any component of growth-promoting system.

The administration of indomethacin (10 mg/kg) to pregnant animals inhibited the

elevation not only of prostaglandins in maternal (99 ± 5 vs 235 ± 29 pg/ml) and fetal (398 ± 152 vs 913 ± 42 pg/ml) circulations, but also of GH (50 ± 5 vs 89 ± 5 ng/ml, and 83 ± 7 vs 137 ± 21 ng/ml, respectively).

In contrast, serum IGF levels in indomethacin-treated pregnant rats were slightly but significantly elevated above those found in control animals throughout gestation. An unexplained dramatic decrease in IGF CP concentrations was observed in both groups of pregnant rats.

This study of the growth-promoting system in growing rats demonstrates its complexity and age dependency. Pregnancy is accompanied by significant and specific changes in all major components of the growth-promoting system. Some of these alterations may be related to elevated levels of PGs while the reasons for the others are still unknown.

The authors wish to acknowledge the expert technical assistance of Nancy A. Sherman, M. S., and Carolyn J. Johnson, B. S., and the careful typing of the manuscript by Ms. Josephine Domingo. Without the generous gift of highly purified IGF by Dr. R. E. Humbel, this work should not have been possible. This work was supported in part by NIH Research Grants AM 18617 and HL 22026.

1. Salmon, W. D., Jr., and Daughaday, W. H., J. Clin. Invest. **35,** 733 (1956).
2. Daughaday, W. H., Hall, K., Raben, M. S., Salmon, W. D., Van den Brande, J. L., and Van Wyk, J. J., Nature (London) **235,** 107 (1972).
3. Zapf, J., Waldvogel, M., and Froesch, E. R., Arch. Biochem. Biophys. **168,** 638 (1975).
4. Megyesi, K., Kahn, C. R., Roth, J., and Gorden, P., J. Clin. Endocrinol. Metabol. **41,** 472 (1975).
5. Moses, A. C., Nissley, S. P., Cohen, K. L., and Rechler, M. M., Nature (London) **263,** 137 (1976).
6. Kaufmann, U., Zapf, J., Torretti, B., and Froesch, E. R., J. Clin. Endocrinol. Metabol. **44,** 160 (1977).
7. Hintz, R. L., and Liu, F., J. Clin. Endocrinol. Metabol. **45,** 988 (1977).
8. Draznin, B., and Schalch, D. S., Clin. Res., **26,** 127A (1978).
9. Ojeda, S. R., Jameson, H. E., and McCann, S. M., Prostaglandins **13,** 943 (1977).
10. Louis, T. U., Stellflug, J. N., Tucker, H. A., and Hafs, H. D., Proc. Soc. Exp. Biol. Med. **147,** 128 (1974).
11. Schalch, D. S., and Reichlin, S., Endocrinology **79,** 275 (1966).
12. Schalch, D. S., Heinrich, U. E., Koch, J. G., Johnson, C. J., and Schlueter, R. J., J. Clin. Endocrinol. Metabol. **46,** 664 (1978).
13. Schalch, D. S., Heinrich, U. E., Draznin, B., Johnson, C. J., and Miller, L. L., Endocrinology **104,** 1143 (1979).
14. Chochinov, R. H., Mariz, I. K., and Daughaday, W. H., Endocrine Society 58th Annual Meeting (Abstract), p. 220 (1976).
15. Rechler, M. M., Zapf, J., Nissley, S. P., Froesch, E. R., Moses A. C., Podskalny, J. M., Schilling, E., and Fryklund, L., Clin. Res., **25,** 300A (1977).
16. Zapf, J., Kaufmann, U., Eigenmann, E. J., and Froesch, E. R., Clin. Chem. **23,** 677 (1977).
17. Moses, A. C., Nissley, S. P., Rechler, M. M., and Short, P. A., Endocrine Society 60th Annual Meeting, p. 89 (1978).
18. Zapf, J., Schoenle, E., and Froesch, E. R., Eur. J. Biochem. **87,** 285 (1978).
19. Hwang, D. H., Mathias, M. M., Dupont, J., and Meyer, D. L., J. Nutr. **105,** 995 (1975).
20. McCosh, E. J., Meyer, D. L., and Dupont, J., Prostaglandins **12,** 471 (1976).
21. Salmon, W. D., and DuVall, M. R., Endocrinology **87,** 1168 (1970).
22. Daughaday, W. H., and Reeder, C., J. Lab. Clin. Med. **68,** 357 (1966).
23. Heinrich, U. E., Schalch, D. S., Koch, J. G., and Johnson, C. J., J. Clin. Endocrinol. Metabol. **46,** 672 (1978).
24. Zapf, J., Schoenle, E., Jagars, G., Sand, I., Grunwald, J., and Froesch, R. E., J. Clin. Invest., in press.
25. Ojeda, S. R., and Jameson, H. E., Endocrinology **100,** 881 (1977).
26. Kaplan, S. L., Grumbach, M. M., and Shepard, T. M., J. Clin. Invest **51,** 3080 (1972).
27. Blazquez, E., Simon, F. A., Blazquez, M., and Foa, P. O., Proc. Soc. Exp. Biol. Med. **147,** 780 (1974).
28. Walker, D. G., Simpson, M. E., Asling, C. W., and Evans, M. M., Anat. Rec. **106,** 539 (1950).
29. Mauger, R. A., and Gorski, J., Endocrinology **101,** 76 (1977).
30. Walker, P., Dussault, J. H., Alvarado-Urbina, G., and Dupont, A., Endocrinology **101,** 782 (1977).
31. MacLeod, R. M., and Lehmeyer, J. E., Proc. Nat. Acad. Sci. USA **67,** 1172 (1970).
32. Hertelendy, F., Acta Endocrinol. **68,** 355 (1971).
33. Ito, H., Momose, G., Katayama, T., Takagishi, H., Ito, I., Nakajima, H., and Takei, Y., J. Clin. Endocrinol. **32,** 857 (1971).
34. Dickerman, E., Dickerman, S., and Meites, J., *in* "Growth and Growth Hormone" (A. Pecile and E. E. Muller, eds.), p. 252. Excerpta Medica, Amsterdam (1972).
35. Merimee, T. J., Burgess, J. A., and Robinowitz, D., J. Clin. Endocrinol. Metabol. **26,** 791 (1966).
36. D'Ercole, A. J., Foushee, B. B., and Underwood, L. E., J. Clin. Endocrinol. Metabol. **43,** 1069 (1976).
37. Gluckman, P. D., and Brinsmead, N. W., J. Clin. Endocrinol. Metabol. **43,** 1378 (1976).

38. Hintz, R. L., Seeds, J. M., and Johnsonbaugh, R. E., Amer. J. Dis. Child. **131,** 1249 (1977).
39. Stuart, M. C., Lazarus, L., Moore, S. S., and Smythe, G. A., Hormone Metabol. Res. **8,** 442 (1976).
40. Saunders, A., Terry, L. C., Audet, J., Brazeau, P., and Martin, J. B., Neuroendocrinology **21,** 193 (1976).
41. Draznin, B., Heinrich, U. E., Chochinov, R., and Schalch, D. S., Clin. Res. **26,** 413A (1978).
42. Daughaday, W. H., and Trivedi, B., Endocrine Society 60th Annual Meeting (Abstract), p. 90 (1978).
43. Daughaday, W. H., and Kapadia, M., Endocrinology **102,** 1317 (1978).

Received January 15, 1979. P.S.E.B.M. 1979, Vol. 162.