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Hormonal and dietary adaptation of rat pancreatic hydrolases before and after weaning

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DESCHODT-LANCKMAN, M., P. ROBBERECHT, J CAMUS, C. BAYA, AND J. CHRISTOPHE. *Hormonal and dietary adaptation of rat pancreatic hydrolases before and after weaning*. Am. J. Physiol. 226(1): 39–44. 1974.—All levels of digestive enzymes did not change in constant proportion in the rat pancreas before weaning, and different stimuli were probably responsible for their emergence. The high levels of amylase and chymotrypsinogen observed during the late fetal period were followed by a marked depression in the newborn period. Prompt administration of hydrocortisone prevented the drop of these hydrolases and enhanced the low activity of trypsinogens in 1- to 23-day-old pups. This observation together with the reported decreasing activity of the pituitary-adrenal axis during the first neonatal weeks suggests that glucocorticoid secretion is a stimulus effective in evoking a series of pancreatic hydrolases. On the other hand, the emergence of lipase was slow, responding poorly to hydrocortisone and to nutritional controls only after 25 days. L-Thyroxine, glucagon, or dibutyryl cyclic AMP was unable to increase the specific activities of all hydrolases tested. Nutritional controls were predominant in regulating levels of hydrolases in adult rats. The time course of variation in specific activities of amylase and lipase on high-corn oil and high-starch diets was unaffected by 7 days of glucagon administration. Adrenalectomy tended to decrease and hydrocortisone treatment tended to increase the level of pancreatic amylase in rats submitted to the same dietary manipulations. The accumulation of amylase required the presence not only of starch but also of protein in the diet.

exocrine pancreas; biochemical differentiation; enzyme adaptation; amylase; lipase; chymotrypsinogen; trypsinogens; hydrocortisone; thyroxine; glucagon; dibutyryl cyclic AMP; dietary carbohydrates; dietary fats; dietary proteins

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THE BIOCHEMICAL DIFFERENTIATION of the liver in relationship to the development of metabolic functions has been well documented and outlined in detail in a recent review (5); however, there have been relatively few observations about the late enzymic differentiation in the exocrine pancreas (11, 14, 18, 25). We have observed that α-amylase and chymotrypsinogen, the best represented hydrolases at birth, are markedly depleted in the rat pancreas within 2–4 days postpartum (13) and that the level of each hydrolase is influenced by dietary manipulations after weaning (2, 13). The purpose of the present report was to differentiate between hormones and nutritional stimuli in the regulation of hydrolases in the rat pancreas before and after weaning. A portion of this study has been presented in abstract form (3).

MATERIALS AND METHODS

All animals were housed in air-conditioned quarters, lighted from 7 A.M. to 7 P.M. Albino rats were fed ad libitum on commercial pellets (UAR, Villemoisson-sur-Orge, France). This standard chow contained (in %, w:w): carbohydrates 50%; proteins 21%; lipids 5%. When indicated, two semisynthetic diets were also utilized. The high-carbohydrate diet was as follows (in %, w:w): starch 66.6%; cow casein (NBC, Cleveland, Ohio) 18.0%; DL-methionine 0.3%; corn oil 4.0%; choline 0.1%; salt mixture USP XIV 4.0%; vitamins diet fortification mixture (NBC) 2.0%; cellulose (Alphacel, NBC) 5%. The high-fat diet was rich in unsaturated fatty acids and very effective in inducing lipase in adult rats (2). This diet consisted of the following: corn oil 50%; casein 38.3%; DL-methionine 0.8%; carbohydrate 0%; choline 0.3%; salt mixture 4.0%; vitamin supplements 2.0%; cellulose 5.0%.

In fetal experiments, 200- to 250-g pregnant rats were used, 4 ± 1 days or 1 day before term. Estimation of fetal ages was based on time matings. Laparotomy was performed under ether anesthesia. Uterine horns were partially exposed and the fetuses were injected intraperitoneally through the uterine wall. Alternate fetuses received 10 μl of proper solvent (controls) or 10 μl of solvent containing either hydrocortisone (250 μg) or L-thyroxine (2 μg). The abdomen was sutured and the fetuses were removed for assays 24 hr later.

In other experiments, birth dates were recorded after twice-daily inspections. Rats were weaned at 21 days postpartum. Since 12-day-old pups begin to nibble at solid cubes, the food offered to the mothers was changed on the 13th day from chow to the high-fat or the high-carbohydrate semisynthetic diets. Pups eating a mixed diet of milk and adult 50% corn oil diet were therefore maintained on a high-fat high-protein diet, whereas those on the other mixed diet had the opportunity to eat large amounts of starch. Newborn and infant rats were randomly selected from the litters at different stages of their postnatal growth and were treated with hormones, cycloheximide, and N^6, O^6-dibutyryl cyclic adenosine 3′,5′-monophosphate as indicated in the appropriate figures and Table 1.

For experiments on adult rats, male animals weighing 180–230 g and previously maintained on the standard chow were used. In a first series of experiments, they were fed ad libitum on the high-corn oil carbohydrate-free diet for 8 days. Food was then withheld for 17 hr after which the ani-

mals were switched to the 67% starch diet for another week. Batches of six rats were killed on *days 1, 2, 5, 8, 9, 10, 13,* and *16.*

The same dietary manipulation was utilized with control rats, rats receiving glucagon intraperitoneally (0.1 mg/100 g body wt, twice daily at 9 A.M. and 5 P.M.), adrenalectomized rats, and adrenalectomized rats receiving hydrocortisone by intraperitoneal injection at a daily dose of 5 mg/100 g body wt. Hormonal treatments never exceeded 7 consecutive days and covered the period corresponding to the last diet administered before sacrifice. Rats submitted to bilateral adrenalectomy 5–10 days before the dietary manipulations were maintained during this postoperative period on standard chow ad libitum. They received 0.9% NaCl as drinking water up to the time of sacrifice.

In a last experiment, the role exerted by protein in a high-starch diet on amylase adaptation was examined in adult animals fed ad libitum for 5 days on the 50% corn oil diet. These rats were fasted 24 h, then force fed 4 times during the next 2 days with starch (2.6 g/100 g body wt) either enriched with or without casein (0.52 g casein/100 g body wt). Batches of six rats were killed at various time intervals.

Fetuses and newborn rats were killed by placing on ice. Infant and adult rats were killed by cervical fracture. The pancreas was quickly removed and stored in the Deepfreeze before assays. This storage was without effect on enzyme activities. Pancreases from three animals were pooled when provided by pups under 8 days of age.

Activation of proenzymes and measurement of enzymatic activities and proteins have been described previously (2, 13). The single-time point saccharogenic assay for α-amylase (23) and the constant pH-titration methods for lipase, chymotrypsin, and trypsins (22) were automated. Enzyme activities were expressed in units, i.e., in micromoles of products liberated per minute at 25 C, and related to 1 mg of protein in the pancreas. Specific activities were considered as a proper mode of comparing the tissue content of one hydrolase relative to another during these developmental studies on the pancreas.

Hydrocortisone acetate (Roussel, Paris, France) and glucagon (purified, Novo Laboratories, Copenhagen, Denmark) were generous gifts. Cycloheximide, L-thyroxine (sodium salt), and N^6,O^6-dibutyryl cyclic adenosine 3′,5′-monophosphate were purchased from Sigma Chemical Co., St. Louis, Mo.

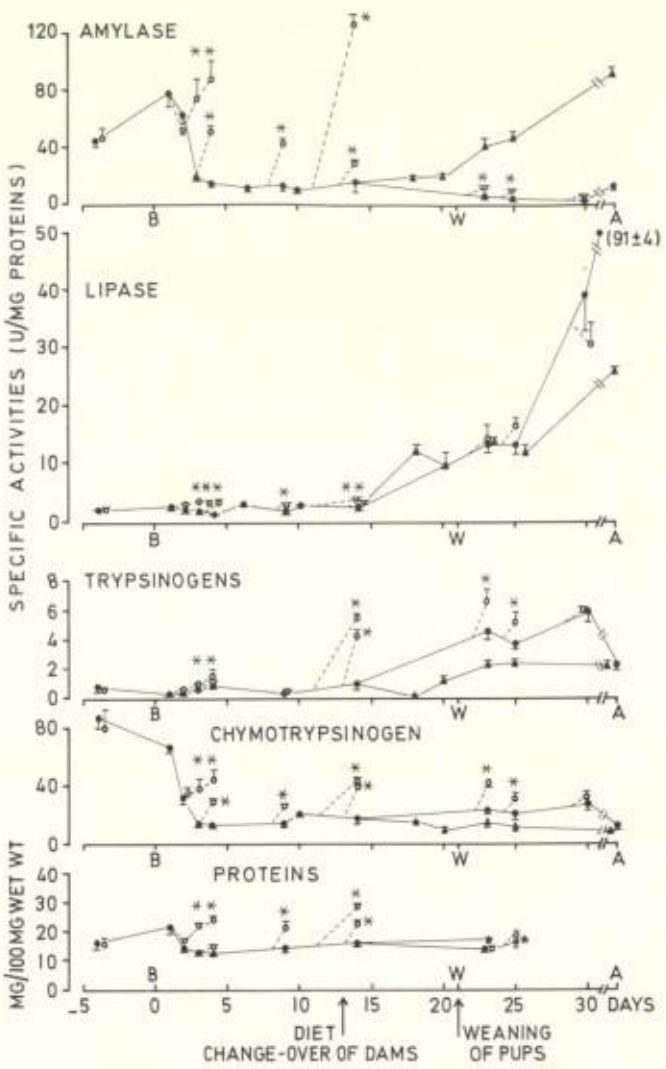

FIG. 1. Comparison of developmental formation of hydrolases in rat pancreas. Broken lines (○ — — — ○) refer to rats given intraperitoneal injections of hydrocortisone daily (5 mg/100 g body wt) and assayed 24 h after last injection. After 14 days postpartum, dams were changed from standard chow to a high-fat (●——●) or high-starch (▲——▲) diet. Each point is a mean of 6 values ± SE. With pups under 8 days of age, each value was obtained on a pool of 3 pancreases. B = birth. W = weaning. A = 180- to 230-g male adults. An asterisk indicates a value significantly different ($P < 0.05$) from that observed without hydrocortisone treatment.

RESULTS

Hormonal controls of pancreatic hydrolases in fetal and neonatal stages of development. The decrease in total proteins observed after birth was followed by a slight gradual upturn after weaning (Fig. 1).

Our new developmental curves of specific activities as a function of age extend those already published by our laboratory (13). The present data (Fig. 1) indicate that amylase and chymotrypsinogen were present in high concentration during the late period of gestation (18th–19th day), whereas lipase and trypsinogens were low relative to activity in adult rats. Amylase and chymotrypsinogen decreased to minimal values 4 days after birth. Weaning rats on the 50% corn oil diet caused the low activity of amylase, characteristic of the suckling period, to persist. The presence of a large quantity of casein in the high-lipid diet could explain the slight increase in trypsinogens around the 3rd week of life (2). The rise of lipase was not faster than in cages where the high-starch diet was offered up to 25 days. After 30 days lipase activity did not reach the levels observed in high-fat fed adult animals ($P < 0.05$ between 30 days and adult rats maintained on this diet). However, these 30-day rats on the high-fat diet had higher lipase than adult rats on the high-starch diet. Hence, high-fat diet did produce a differential effect for lipase after 25 days of age.

Single intrafetal injections of hydrocortisone (Fig. 1) and L-thyroxine (Table 1) were without effect on enzymatic specific activities and protein concentrations 24 hr after treatment.

TABLE 1. *Effects of L-thyroxine, glucagon, and dibutyryl cyclic AMP*

n	Treatment and No. of Intraperitoneal Injections	Age at Time of Sacrifice, days	Proteins	Amylase	Lipase	Chymotrypsinogen	Trypsinogens
4	Controls	0	13.4 ± 1.8	102.1 ± 11.2	2.34 ± 0.06	49.8 ± 3.2	0.38 ± 0.11
5	L-thyroxine (×1)	0	16.1 ± 1.3	104.2 ± 5.4	2.45 ± 0.14	53.7 ± 2.4	0.28 ± 0.12
12	Controls	2	14.1 ± 1.0	62.1 ± 6.4	1.74 ± 0.20	31.2 ± 3.2	0.37 ± 0.05
3	L-thyroxine (×1)	2	12.2	55.4	1.25	19.1	0.34
2	Glucagon (×1)	2	17.3	54.6	1.60	31.3	0.36
2	DB-cAMP (×1)	2	14.0	52.2	1.53	37.2	0.56
11	Controls	3	12.2 ± 0.3	18.1 ± 2.6	1.32 ± 0.08	13.8 ± 0.8	0.63 ± 0.06
5	L-thyroxine (×2)	3	9.9 ± 0.3	18.5 ± 1.6	0.59 ± 0.10	6.0 ± 0.7	0.25 ± 0.09
2	Glucagon (×2)	3	11.0	12.1	1.33	12.6	0.73
2	DB-cAMP (×2)	3	10.7	12.8	1.33	16.1	1.07
17	Controls	4	11.8 ± 0.4	13.2 ± 0.8	0.91 ± 0.06	12.5 ± 0.8	0.84 ± 0.09
3	L-thyroxine (×3)	4	11.0	9.3	0.45	5.8	0.02
4	L-glucagon (×3)	4	12.9	12.0	0.88	12.0	0.92
3	DB-cAMP (×3)	4	11.5	8.3	1.24	19.0	1.07

Changes in specific activities of digestive enzymes (U/mg proteins) and protein content (mg/100 mg wet wt) in rat pancreas were recorded during the late fetal and neonatal periods. All animals were given daily intraperitoneal injections of L-thyroxine (2 μg), glucagon (50 μg), or dibutyryl cyclic AMP (DB-cAMP: 125 μg). All assays were conducted 24 h after the last injection. Within pregnant rats alternate 20- to 21-day-old embryos were injected with solvent only as indicated under methods and served as controls. Values are means ± SE. Each value was obtained on a pool of three pancreases. *n* = Number of experiments.

Newborn rats, treated on *days 1, 2,* and *3* with glucagon (50 μg daily) or dibutyryl cyclic AMP (125 μg daily), failed to respond. During this neonatal stage of development, the daily injection of 2 μg of L-thyroxine led to a decrease in specific activities of all hydrolases except amylase (Table 1). On the other hand, injections of hydrocortisone maintained high levels of amylase as well as chymotrypsinogen in 3-day-old pups (Figs. 1 and 2). Hydrocortisone was also able to trigger the appearance of slightly (but significantly) higher amounts of lipase and trypsinogens. Cycloheximide, when administered with hydrocortisone (Fig. 2), partially prevented glucocorticoid effects. (Higher doses could theoretically be more effective in inhibiting protein synthesis, but in practice such toxic doses were lethal within 24 hr.)

At the end of the 2nd week, the increase of amylase, chymotrypsinogen, and trypsinogens under hydrocortisone treatment was of great magnitude (Fig. 1). Although administration of hydrocortisone was still able to enhance these activities at 23 days, its effect was less obvious 2 days later and no longer significant at 30 days.

Hormonal and dietary controls of pancreatic hydrolases in adult rats. Figure 3 illustrates the time course of variations in specific activity of amylase and lipase in adult rats fed successively on high-fat and high-starch diets. A comparison was made between untreated animals, adrenalectomized rats, and adrenalectomized rats treated with hydrocortisone. The kinetics during the two consecutive 7-day periods were always those of exponential adjustments.

In control animals, large increases and decays in specific activities developed during short-term dietary adaptations, but the half-life of these adaptations remained approximately the same (2 days). The opposite variations of amylase and lipase imply important and divergent alterations in the relative rate constants of biosynthesis (17).

When compared to these control values, amylase levels in adrenalectomized rats tended to be low throughout the experimental period. However, the response to dietary manipulations was not basically obliterated, and the $T_{1/2}$ of adaptations remained similar. Hydrocortisone treatment of adrenalectomized rats slightly depressed lipase levels.

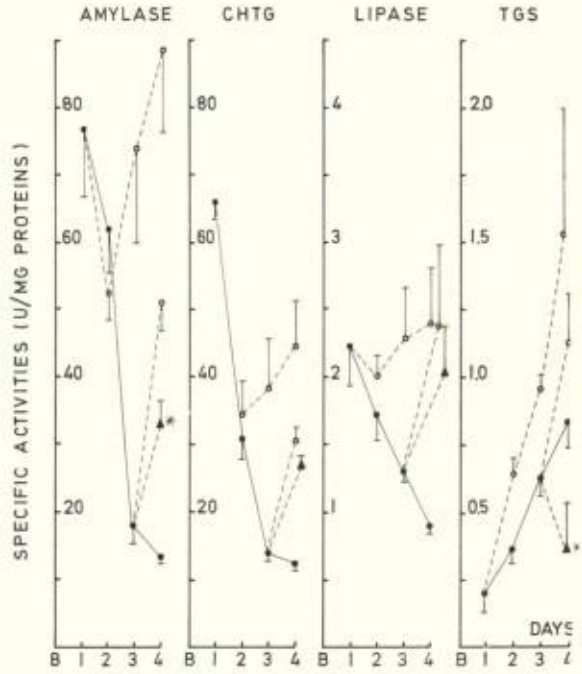

FIG. 2. Pancreatic hydrolases in control animals (●) and after treatment with hydrocortisone (○) and hydrocortisone plus cycloheximide (▲) during early postnatal period. Cycloheximide (100 μg/100 g) and hydrocortisone (5 mg/100 g) were injected intraperitoneally. Broken lines (– – –) indicate duration of treatments. Means of 6 determinations ± SE. Each value was obtained on a pool of 3 pancreases. Chtg = chymotrypsinogen. Tgs = trypsinogens. B = birth. An asterisk indicates a value significantly different from that obtained without cycloheximide.

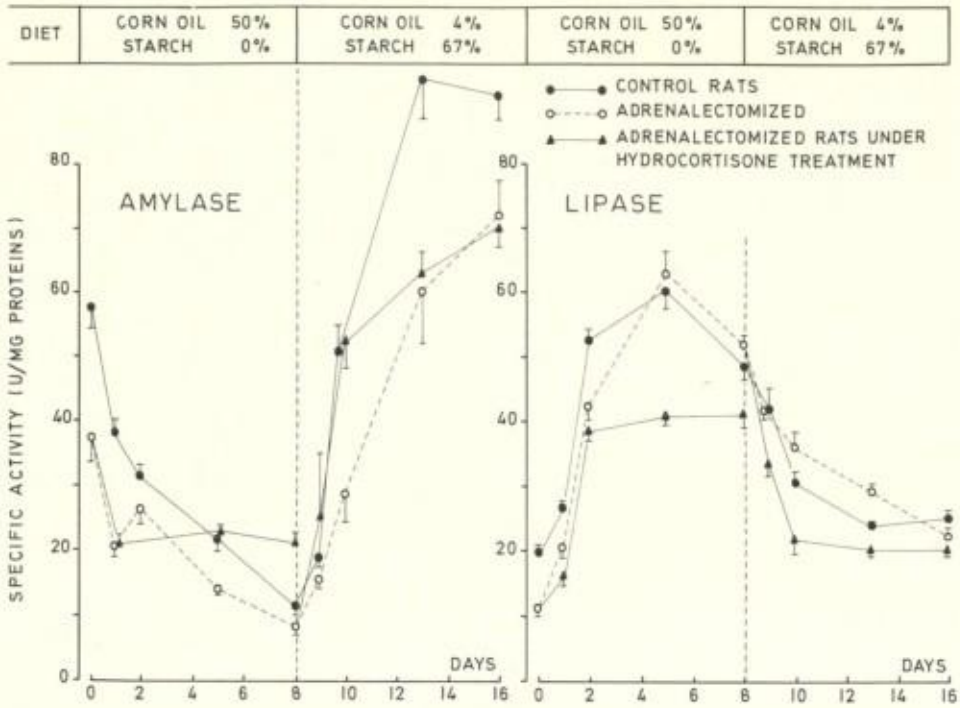

FIG. 3. Development of activity of amylase and lipase in pancreas of adult rats. Figure shows time course of induction, with either high-fat or high-carbohydrate diet. Normal rats (●——●), adrenalectomized rats (○ – – –○), adrenalectomized rats treated with hydrocortisone (▲——▲) (5 mg/100 g daily starting on *day 0* or *day 8* of experiment). Means of 6 determinations ± SE.

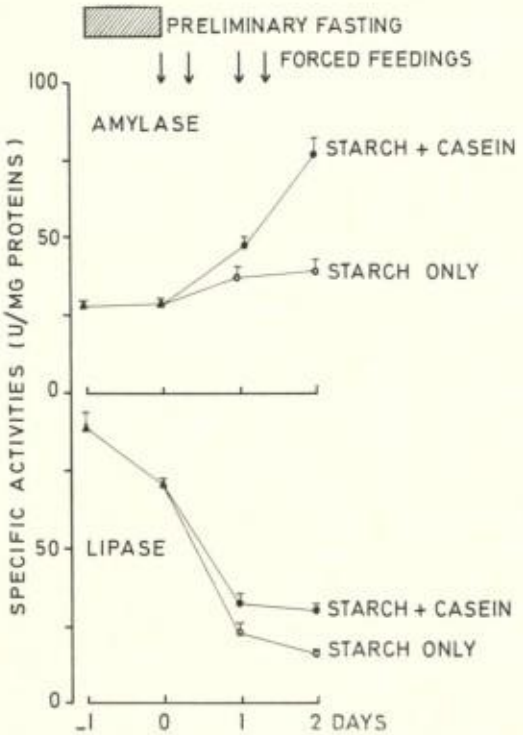

FIG. 4. Permissive role of casein in amylase induction obtained on starch in adult rats. Animals fed ad libitum for 5 days on a 50% corn oil diet were fasted 24 hr (▲) and then force fed 4 times (↓) during next 2 days with starch (2.6 g/100 g body wt), either enriched with (●) or without (○) casein (0.52 g/100 g body wt). Batches of 6 rats were killed at 4 time intervals. Means of 6 determinations ± SE.

In parallel experiments, using exactly the same time schedule of dietary manipulations, the administration of glucagon for 7 days was without effect on amylase and lipase adaptations in normal rats (data not shown).

In force fed rats previously maintained on a high-corn oil diet, amylase induction by starch was observed only in the presence of casein offered as a dietary protein of high quality (Fig. 4). This adaptation was as efficient as that observed when a complete high-starch diet was fed ad libitum (Fig. 3). On the other hand, the depression in lipase specific activity did not depend on the presence of casein.

DISCUSSION

Enzyme activities reflect tissue contents in the case of pancreatic hydrolases, and changes in content result from protein synthesis or secretion.

Enzymic differentiation in exocrine pancreas during late fetal and neonatal periods. Rutter et al. (14) have shown that there is an inflexion point in the development of the exocrine pancreas at 17–18 days of gestation, with a striking increase to the high levels of some hydrolases found in the 20- to 21-day-old embryo. This phenomenon parallels an increased secretory activity of the endocrine pancreas, the pituitary, the adrenal cortex, and the thyroid during the late fetal period (references in 5). High specific activities of pancreatic hydrolases at birth could not only reflect high rates of synthesis (derepression), but also low rates of secretion (13). The ability to secrete amylase in the presence of secretagogues is indeed increasing with developmental age (8).

At variance with Rutter et al. (14) and Corring and Aumaitre (1), we found an important lag in trypsinogens and lipase accumulation in 18- to 19-day-old rat fetuses. The evolution of enzymic patterns during the prenatal and neonatal periods was a discontinuous process. All levels did not change in constant proportion (Fig. 1), and different stimuli were probably responsible for the emergence of each hydrolase.

The apparent purposefulness of differentiation among

pancreatic proteases after birth would be better understood if we knew how the rat stomach and small intestine are ready to cope with maternal milk and solid food offered after weaning. The delayed occurrence (Fig. 1) of pancreatic trypsinogens together with the presence of inhibitors from colostrum, active against trypsin and chymotrypsin (9), could allow the absorption of maternal immunoglobulins from the gastrointestinal tract during the 1st days after birth, especially if gastric pepsinogens were also inactive.

Gastric and milk lipases could substitute in suckling rats for the digestive function assumed by pancreatic lipase in adult animals. Indeed, a gastric lipase with a pH optimum about 5 exists which preferentially hydrolyzes triglycerides with short- and medium-chain fatty acids. Since the pH of the gastric mucosa is about 4 in 10-day-old rats and since one-fourth of rat milk triglycerides contain less than 14 carbon atoms, Helander and Olivecrona (6) have suggested that gastric partial lipolysis could play an important physiological role before weaning, i.e., before significant pancreatic lipase induction (Fig. 1).

Hormonal adaptation before and after weaning. The exocrine pancreas of fetal rats failed to respond to hydrocortisone. It may well be that the level of endogenous glucocorticoids was high enough in the rat fetus for pancreatic differentiation, since in an embryonic chick pancreas cultured in vitro the rate of accumulation of amylase is greatly increased by hydrocortisone addition (25). Immediately after birth the pituitary adrenal axis is very responsive to starvation, but becomes inactive after a few days (5). The function of this system is reestablished after 2 weeks of extra-uterine life (5).

One of the reasons that the administration of hydrocortisone caused amylase, chymotrypsinogen, and trypsinogens formation in 1- to 25-day-old rats but not in animals aged 30 days (Figs. 1 and 2) may be that the adrenals resumed a high rate of glucocorticoid secretion after 1 month. A striking increase to high levels of amylase, proteases, and zymogen granules has already been demonstrated in 9-day-old pups treated with cortisone (11, 18). Between 15 and 30 days of age the size of acinar cells and pancreatic protein concentration increase markedly and spontaneously (1, 19).

The basis for the action of hydrocortisone in regulating enzyme levels in the pancreas before weaning is not known. Hydrocortisone provokes allosteric changes in nucleolar RNA polymerases, enhances ribosomal RNA synthesis, and induces a cyclic AMP-dependent protein phosphokinase in hepatic cells (15, 16).

A stimulus effective in the neonate is not necessarily operating as obviously later on.

Hydrocortisone only played a limited role in regulating hydrolases in adult rats, and stimulated protein breakdown under the influence of this hormone might have exerted additive indirect effects under our experimental conditions. Snook (21) and Sesso et al. (20) had already seen that adrenalectomy decreased pancreatic amylase (and RNA), whereas lipase was maintained at normal level. We obtained similar data and demonstrated that hydrocortisone was not required for dietary adaptation of amylase and lipase (Fig. 3).

Repeated injections of glucagon for 1 week failed to influence the levels of amylase and lipase in adult rats. This was unexpected since other authors have reported a degranulation of the rat pancreas (7) under chronic treatment by this short-lived hormone and a depression of secretion by a single injection of glucagon in the dog pancreas stimulated by pancreozymin and secretin (4).

Dietary and hormonal intricacy of amylase adaptation in weanling and adult rats. In the 3rd week of life, dietary changes associated with weaning exerted positive or negative influences on amylase levels. The developmental accumulation of amylase occurring on transition from milk to the usual solid high-carbohydrate diet was in clear contrast with the inhibition observed on the high-corn oil diet (Fig. 1). A similar observation has been made with hepatic glucokinase (24). This lack of accumulation on the high-corn oil diet was probably linked to low-insulin secretion. Insulin secretion under the influence of the high-starch diet also contributed to enhance amylase levels in adult rats (Fig. 3 and refs. 2, 10, 12, 21). Casein permitted amylase accumulation (Fig. 4), but was insufficient per se for maintaining high levels on a high-fat carbohydrate-free diet (Fig. 3 and ref. 2). In conclusion, the appearance of amylase was dependent on three stimuli acting in conjunction: starch, protein, and insulin.

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