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DEVELOPMENT OF AVIAN LIVER LIPOGENIC ENZYMES DURING THE PERINATAL PERIOD. MINIREVIEW.

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Contrary to the mammalian embryo, lipid synthesis is minimal in the liver of the chick embryo. During this period, the animal is consuming a fatty diet from where it derives its energy in the last part of its embryonic life. It is understandable that there is no necessity to elaborate these enzymes, since the embryo environment is rich in lipids. After hatching, a high carbohydrate-low fat diet is installed and the animal has to adjust to this new nutritional state developing the hepatic lipogenic activity with a concomitant coordinate increase of the enzymes involved in this pathway.

It has been shown (5, 7) that the activity of **citrate lyase** (CL) increases slightly but significantly during the hatching period and the starvation period following hatching. After 24 h of feeding, the values are elevated 4.5 fold reaching the maximum activity 5-6 days after feeding. The **fatty acid synthetase** (FAS) activity, as found by Joshi and Sidbury (14) in the newly hatched chicks, goes up even if the animal is starved. After feeding, there is an additional increase. The maximum activity is found at 2-4 days, going down to adult values in ten days. **Malic enzyme** (ME) behaves like the other two enzymes (7), however there is no increase in activity before feeding is established. We showed (24) at the same time as did Arinze and Mistry (1), that the **acetyl CoA carboxylase** (ACCx) activity is very low during the embryonic period. During the hours after hatching there is a rise and with the onset of feeding the activity increases 8-10 times, peaking at 15 days. This enzyme is activated by citrate, and at any point there was a citrate requirement (5mM) for maximal activity (26). It seems then

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that CL, ACCx and FAS share a common characteristic, not seen with ME which is that the elevation in activity observed during hatching period is independent of feeding.

The first question is to ascertain if this increase in activity was due to activation of preexisting enzyme molecules or to synthesis of new protein. Our first approach was to determine the rate of holoenzyme formation using the ATP-dependent C^{14} biotin fixation to a 25% ammonium sulfate fractionated enzyme from chick liver cytosol at different stages of development. This showed that with hatching there is an important increase in holoenzyme formation (25). It implies then, that with development, there was an increase of the apoprotein. Presence of holoenzyme synthetase during the whole period and of the coenzyme is likely because Arinze and Mistry have demonstrated (1) high activities of other biotin enzymes, and it is assumed that the same holoenzyme synthetase is active for all biotin enzymes (20).

Later on, studies in different laboratories (28, 29) of rate of synthesis of some of these enzymes during hatching and subsequent days have revealed that in every case, increased activity corresponds to increase rate of synthesis.

Several agents have been considered are effectors of this increase: drastic environmental change, coenzyme levels, metabolites and hormonal factors. Let us consider each one of these.

1. **Drastic environmental change.** Placing eggs in electric forced incubators and flushing continously with 100% oxygen, or air, Goodridge found (8) that the activity of FAS increased 1.5 times while ACCx was not affected.

2. **Coenzyme levels.** Birnbaum in 1969 (2) described a mechanism of coenzyme repression observed in **L. plantarum**: the ACCx was inhibited when the bacterial cells were grown in excess biotin. In 1971 there is a report (19) where the authors claim finding a similar phenomena of repression of ACCx for biotin, in rat adipose tissue. The liver enzyme in their experiments showed the opposite effect: it was induced by the coenzyme.

The biotin concentration in egg yolk is quite high (500 mμg/g) (9). We determined the biotin concentration in chick liver at three different developmental stages and found (26) a notable decrease in free biotin content of chick liver cytosol after hatching, following an inverse course to the appearence of the carboxylase (Fig. 1). Despite this, the coenzyme does

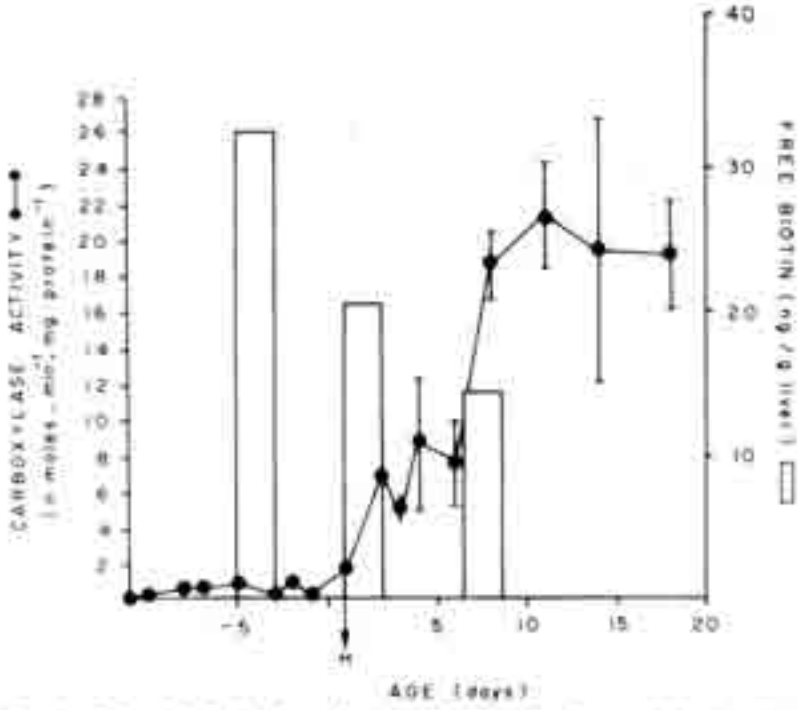

Fig. 1.– Free biotin levels and acetyl CoA carboxylase activity in chick liver cytosol during development.

not seem to play any role in the synthesis of the enzyme protein since in animals injected with biotin, the activity after seven days, was the same as in the control group (26). Although we observed a small change in the amount of protein precipitable at 25% ammonium sulfate, the amount of immunological precipitable protein per unit of enzyme activity was unaltered.

3. **Metabolites.** Goodridge in 1973 (9) tried to identify intermediates which might be potential regulators of the concentration of lipogenic enzymes. He found that glucose injections in neonatal chicks stimulate fatty acids synthesis and the total activities of ACCx, FAS and ME. He also found a decrease of free fatty acids and fatty acyl CoA in the liver. He concluded that glucose (or a metabolite of it) is the natural inducer or corepressor for the synthesis of the lipogenic enzymes.

He determined the concentration of other metabolites such as acetyl CoA and citrate, not finding any relationship between their levels and enzyme activities (9). Rinaudo and co-workers (23) have reported that citrate levels go down in the liver tissue with development; the same situation is observed with ATP levels, while AMP went up continously.

An important phenomenon observed in chicks during the first hours after hatching is the elevation in blood glucose (13, 21, 22), which is accompanied by a marked depletion of the hepatic glycogen content (22). Rinaudo and co-workers (22) noted that the glucose level at the liver cell goes up slowly during development and Raheja et al. (21) showed that plasma glucose increases during the fasting period that follows hatching, and that after feeding is started, an additional increase occurs.

We studied different conditions altering the blood glucose levels in one day-old starved chicks, and found that although the glycemia suffered important changes, there was no correlation with the activity of acetyl Coenzyme A carboxylase (27) (Fig. 2).

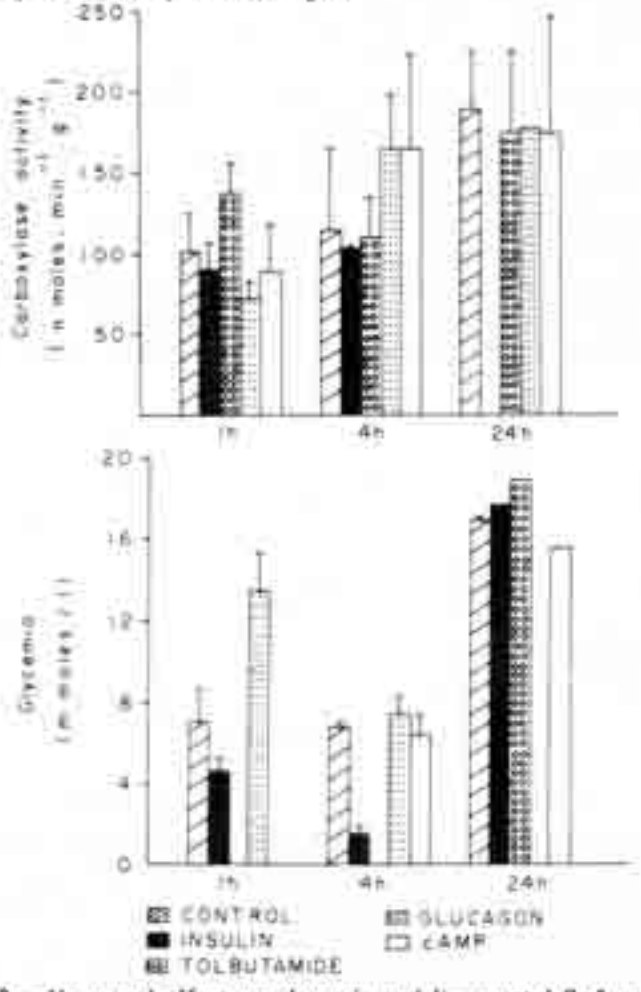

Fig. 2.— Hormonal effect on glycemia and liver acetyl CoA carboxylase activity in unfed newly hatched chicks.

4. **Hormonal effect.** The introduction of different hormones failed to produce "in vivo" any induction of the ACCx activity in newly hatched chicks (27). Studies with embryos revealed the same situation.

Goodridge has been able to induce ME in isolated hepatocytes in culture from 18-day-old embryos with thyroid hormones (triiodotyronine being more active than thyroxine) and with insulin (11). No "in vivo" induction of ME has been reported. Joshi and Sidbury in 1976 (10) showed a brief premature induction of FAS in 20-day-old embryos with "in vivo" injections of several hormones as insulin, glucagon and cyclic AMP, depending on the time of application. Recently (18) he suggested that the glucagon effect is due to an increased level of insulin provoked by the glucagon injection. The same effectors were active "in vitro".

In the case of ACCx, in vivo studies have not been successful in promoting induction with any hormones tested (15, 27). Besides the hormones that effect blood glucose levels, we tested other hormones with no success (27). However, due to the limited effect observed four hours after the injection of glucagon, and based on a very brief precocious rise observed by Goodridge on CL and FAS in his hepatocyte system (10), we decided to check the effect of glucagon on the carboxylase in "in vitro" systems. Working with embryo liver explants or isolated cells mantained in culture for 40-72 hours, we found in each of the mentioned systems, an evident effect of glucagon on the activity of ACCx (Fig. 3) (Ryder, unpublished results).

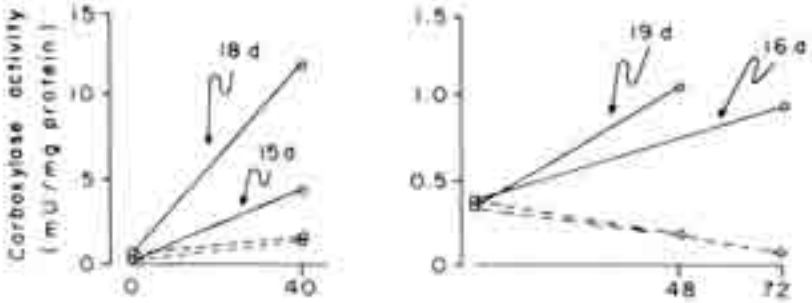

Fig. 3.– Effect of glucagon on acetyl CoA carboxylase activity in livers from 15-19 days old chick embryos, in culture. 1-2 x 10^6 hepatocytes isolated by trypsinization of the tissue or 10-20 one mm^3 liver explants over culture grids were placed on 5-cm culture dishes containing 5 ml of MEM with Hanks salts and L-glutamine, 25 mM HEPES buffer, 10% chicken serum and the antibiotic mixture. Incubated at 37° on humidified incubator under air. ○——○ Control; ○——○ 1 μg/ml glucagon.

Recently, Goodridge and Fisher claimed to find stimulation of ACCx synthesis (and of FAS) in their hepatocytes with triiodothyronine plus insulin, an effect that was blocked by glucagon (12).

In adult animals, changes in the total activities of CL, ME, ACCx and FAS, which occur after nutritional or hormonal manipulations, are usually coordinate. Total activity changes are due to variations in the rate of synthesis; consequently control of synthesis of two or more enzymes could be associated with an operon or its eukaryote equivalent. However, it seems that lipogenic enzymes, when they first appear during hatching, behave differently.

In considering the reported levels of hormones, which are thought to be effective inducers of lipogenic enzymes during development the following picture emerges.

Thyroid hormones: It has been reported that the T4 levels are high on the 17 embryonic day, declining until hatching and the following week, with an only transient increase on day one (4). Other authors (20) showed a completely different picture: T4 increases during development with its highest point on day 19-20 and decays abruptly on day 21 (hatching), while T3 increases continously in the post-hatching period.

Pancreatic hormones: Plasma insulin levels during development have been reported only for hatching period and for subsecuent days (21). It does not change significantly during the post-hatching fasting period; the increase in plasma glucose attributable to the hepatic glycogen depletion (22) is perhaps promoted by glucagon. Glucagon levels have not been reported. The insulin level increases significantly only one day after feeding starts (21).

In Hazelwood's laboratory it has been found (18) that "in vitro" the level of glucose necessary to stimulate insulin release from chicken pancreas over the basal rate is 500-700 mg/dl. With non stimulating levels of glucose, glucagon increases two-fold the release; and tolbutamide, produced a two-fold rise at glucose concentrations of 225 mg/dl.

Insulin effects in the regulation of carbohydrate and lipid metabolism in chicks are uncertain since these animals are resistant to diabetogenic agents and their metabolism is quite different from mammals. One interesting fact is that insulin and glucagon, both elevate liver cyclic AMP levels and free fatty acids (6). Glucagon has been considered more important than insulin in avian metabolism.

The situation at the moment is controversial. Some lipogenic enzymes have been induced "in vivo" and insulin seems to be the hormonal effector. Others, have only been induced using "in vitro" systems; in these cases there was a synergistic effect of thyroid hormones plus insulin. On the other hand, glucagon might be an effective inducer too, and probably not only through its effect on insulin levels. Inhibition of some of these hormones might bring some light into this situation. Radiothyroidectomy or goitrogen treatment of embryos could prevent the inductive action of thyroid hormones. The difficult would arise with insulin: it has been impossible to render chicks diabetic, alloxan or streptozotocin have failed to do it. The use of anti-insulin serum might be of some help (17).

Work is in progress to asses the importance of these hormones on the appearence of the ACCx in newly hatched chicks; to verify if there is a coordinate induction of all lipogenic enzymes evoked by the same hormonal effectors, and to determine whether the same effectors are active in the synthesis of enzyme protein during the first upsurge during development as in adult animals after hormonal or nutritional changes.

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RESUMEN

Desarrollo de enzimas lipogénicas hepáticas de aves durante el período perinatal. Revisión. *Ryder E., Campos G. (Instituto de Investigaciones Clínicas, Facultad de Medicina, Universidad del Zulia, Apartado 1151, Maracaibo, Venezuela).* **Invest Clín** *20(3): 173-187, 1979.*— La actividad lipogénica hepática en las aves comienza inmediatamente después del nacimiento y se acompaña de un aumento coordinado en la actividad de las enzimas lipogénicas: citrato liasa (CL), acetil CoA carboxilasa (ACCx), sintetasa de los ácidos grasos (FAS) y enzima málico (ME). Estas actividades aumentan en ausencia de cualquier cambio dietético y parece ser independiente de la concentración de la glucosa plasmática, de los niveles de coenzima o de los moduladores, lo que sugiere que la alimentación incrementa, pero no inicia la expresión de los genes. Se ha establecido que estos cambios de actividad son debido a síntesis de nueva proteína y no a activación de enzimas preexistentes en forma inactiva. Sobre algunas de estas enzimas se ha demostrado efectos hormonales. Estudios "in vitro" han mostrado que la insulina y la tiroxina incrementan la actividad, y la cantidad de proteína precipitable inmunológicamente, de la

ME, ACCx y FAS en cultivo de hepatocitos de embrión de pollo. Este efecto es bloqueado por el glucagon. La glándula tiroidea ha estado implicada en la iniciación del proceso del nacimiento del pollo, pero los reportes sobre los niveles de hormonas tiroideas circulantes durante el desarrollo son contradictorios. Los niveles de insulina durante el período perinatal no parecen variar si el animal no es alimentado, al menos durante los dos primeros días que siguen al nacimiento. Mas aún, su efecto en la regulación de los metabolismos de carbohidratos y lípidos en las aves es todavía incierto, ya que estos animales son resistentes a los agentes diabetogénicos. Los estudios "in vivo" solamente han conseguido producir una inducción breve de la actividad de FAS por la acción de insulina, glucagon, AMP cíclico y otras hormonas, dependiendo del momento de la aplicación del inductor.

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