Protein Synthesis Regulation by Arginase in Chicken Liver[1]

In the chicken liver of the New Golden Leghorn strain fasting promotes the appearance of a new arginase activity. Under the same conditions, the incorporation of labelled arginine into the liver protein is severely decreased, the decrease being directly related to the levels of the new arginase activity. This could represent a mechanism of regulation of protein synthesis and allow the animal to spare the energy supply in all the conditions where food is scarce.

The chicken, either fasting or fed, usually has a low liver arginase activity[2-4]. However, in some strains, such as the New Golden Leghorn (NGL), arginase activity is increased up to 20-fold by fasting or by a high protein diet, and returns to normal by refeeding for 24 h with the normal diet. This increase is caused by the appearance of a new type of arginase (arginase B) which differs from that present in the fed animals (arginase A) for the chromatographic behaviour, for a lower sedimentation coefficient and for a lower Km for arginine (7 mM) compared to 92 mM for arginase A[5,6].

The dramatic increase of the total liver arginase activity and the lower Km for arginine could affect the steady state concentration of this amino acid in the cell and therefore influence its metabolic processes. In this paper it is shown that, after fasting, the incorporation of arginine into the liver protein of NGL chickens is severely decreased, the decrease being related to the levels of liver arginase activity.

Materials and methods. New golden leghorn and white leghorn chickens of 2 to 3 weeks old were purchased from the Stazione Sperimentale di Pollicultura, Rovigo (Italy). Arginase activity was assayed as previously described[5]. A unit was defined as the amount of enzyme which catalyzes the formation of 1 µmole of ornithine per hour at 37 °C. Liver cell suspensions were prepared in Krebs-Ringer-bicarbonate medium as described by GUDER et al.[7] and contained 1 g of cells per 5 ml of suspension. For the determination of the intracellular arginine and leucine content at the beginning of the experiment, liver cell suspensions were homogenized with 4 volumes of 8% perchloric acid and the amino acid content was determined on the neutralized perchloric acid extract according to SPACKMAN et al.[8].

Results. It was found that leucine incorporation was 3-fold lower and arginine incorporation 9-fold lower in the liver of the 48 h fasted animal (1740 arginase units, Km for arginine = 7 mM, per g of fresh tissue) than in the liver of the fed control (36 arginase units, Km for arginine = 92 mM, per g of fresh tissue) (Figure 1). Furthermore (Table) the incorporation of ^{3}H-leucine and particularly of ^{3}H-arginine was inversely related to the levels of arginase activity. The process of the incorporation of ^{3}H-arginine into the liver protein was saturated in the fed animal (60 arginase units per g of liver) at a lower concentration of arginine than in the 48 h fasted animal (2000 arginase units per g of liver). The overall apparent Km for arginine, comprehensive of all the metabolic processes, from the penetration of the

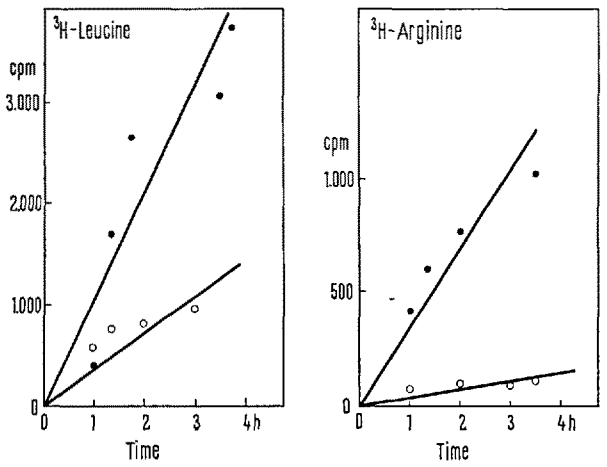

Fig. 1. Incorporation of ^{3}H-leucine and ^{3}H-arginine into the liver protein of fed and fasted NGL chickens. The incubation mixtures (1.5 ml) contained 0.1 g of the liver cells suspension from a fed (●) or from a 48 h fasted chicken (○); in Krebs-Ringer-bicarbonate medium containing 8 mM glucose and all the amino acids at a concentration of 0.33 mM each. Of the amino acids, either leucine, specific activity 3548 cpm per nmole (left side of the figure); or arginine, specific activity 1920 cpm per nmole (right side of the figure) were labelled. The pH was 7.4, temperature was 38 °C. At the times indicated in the figure, the reaction was stopped by the addition of 5 ml of 8% trichloriacetic acid and the protein precipitate was prepared for counting according to SIEKEVITZ[9], dissolved in 60% formic acid and the radioactivity determined in a Tri Carb Liquid Scintillation Counter.

The relation between amino acids incorporation and arginase activity

	Amino acid incorporation per 100 mg of cells		Arginase activity (units/g of liver)
	^{3}H-Leucine cpm	^{3}H-Arginine cpm	
Fed chicken	1700	670	53
24 h fasted chicken	960	260	580
48 h fasted chicken	700	100	1900

The incubation mixtures, prepared as described under Figure 1, contained all the amino acids at 0.33 mM concentration. Of the amino acids either leucine, specific activity 3548 cpm per nmole, or arginine, specific activity 1920 cpm per nmole, were labelled. After 1 h of incubation at 38 °C and pH 7.4, the reaction was stopped by the addition of 5 ml of 8% trichloroacetic acid and the protein precipitate was prepared for counting and counted as previously described. Arginase activity was assayed as described under methods.

[1] Supported by grants from the Italian Consiglio Nazionale delle Ricerche (Impresa di Enzimologia) and from the National Institutes of Health No. GM 12291.

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arginine of the medium into the cell to the incorporation into the protein, was 0.1 m*M* in the fed animal and 0.6 m*M* in the fasted animal (Figure 2).

Discussion. The incorporation of ^{3}H-arginine into the liver protein of fasted chickens of the NGL strain is decreased, as compared with the fed controls, to a larger extent than that of ^{3}H-leucine. This does not occur in other strains, such as the white leghorn: there fasting is not followed, as is typical for the NGL strain, by the large increase of an arginase activity with low Km for arginine.

Many factors could influence the labelling of the liver protein by ^{3}H-leucine and ^{3}H-arginine. First, a larger isotopic dilution in the fasted as compared with the fed animal could be the reason. This, however, is not the case. In fact, at the beginning of the experiment, the intracellular leucine content was 9 nmoles per 100 mg of liver in the fasted and 6.2 nmoles in the fed animal, while the arginine content was 9.2 nmoles per 100 mg in the fasted and 4.1 nmoles in the fed animal. These differences are not important since the equilibration between the extra- and the intracellular amino acids is rapid[10–12] and the amount of the added labelled leucine and arginine was much in excess (0.5 nmole) of that present in the cells.

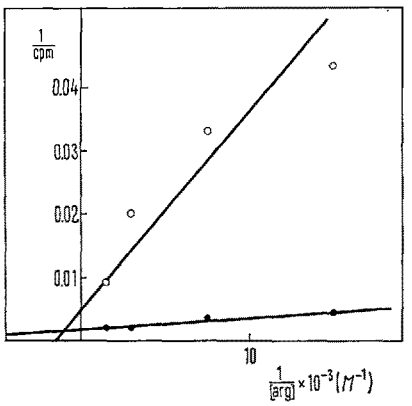

Fig. 2. Incorporation of ^{3}H-arginine in the liver protein of fed and 48 h fasted NGL chickens as a function of arginine concentration. The incubation mixtures were prepared as described under Figure 1. The concentration of ^{3}H-arginine (specific activity 960 cpm per nmole) was: 0.66, 0.33, 0.132; 0.06 m*M*. Temperature was 38 °C, pH was 7.4. After 1 h of incubation the reaction was stopped by the addition of 5 ml of 8% trichloroacetic acid and the protein precipitate was prepared for counting and counted as described under Figure 1.

Fasting could slow down the protein synthesis by decreasing the concentration of unknown factors. This, however, could hardly explain why, under the same experimental conditions, arginine is incorporated to a lesser extent than leucine.

It seems, on the contrary, reasonable to relate the larger decrease of arginine incorporation to the higher level of the liver arginase activity of the fasted NGL chickens (Figure 1, Table). This conclusion is also supported by the observation that the effect of fasting on arginine incorporation into the liver protein can be almost completely removed by increasing the arginine concentration (Figure 2).

The arginase level can thus control, in the liver of the chickens of the NGL strain, the rate of arginine incorporation and therefore the synthesis of protein, particularly of the basic protein such as histones. On the contrary, synthesis of the acidic protein, such as arginase from chicken liver, should be influenced to a lesser extent. The proposed mechanism of regulation could represent, in all conditions where food is scarce, a useful mechanism to spare the energy supply and thus to provide a survival advantage to the animal.

Riassunto. La comparsa, da digiuno, di una nuova attività arginasica nel fegato di pulcino del ceppo Nuova Livornese Dorata, è associata al decrescere della incorporazione di arginina nelle proteine epatiche. Il fenomeno potrebbe avere interesse nella regolazione della sintesi proteica.

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Prostaglandins and Food Intake of Rats

To our knowledge, the effects of prostaglandins (PG) on food intake have not been reported. Because PG are ubiquitous and have been shown to have many effects on physiological and biochemical functions which play some part in the regulation of energy balance, it is conceivable that they may be a component of the food intake regulatory system.

PG are synthesized in various organs including stomach[1], intestines[2], adipose tissue[3], nerves[4,5], and brain[6]. Of the many effects of PG, those which are related to the control of food intake include: stimulation of gastrointestinal motility[7], inhibition of gastric secretion[8], and inhibition of lipolysis caused by epinephrine, norepinephrine, ACTH, TSH, glucagon and growth hormone[9]. PGE_1 has insulin-like activity in that it increases glucose uptake by adipose tissue and stimulates synthesis of triglycerides from glucose and acetate[10,11]. Also BERGSTRÖM et al.[9] have suggested PG have a role in the control of free fatty acid mobilization. Although the above mentioned physiological and biochemical processes are related to some aspect of the control of food intake, it is not apparent what the net result of any one PG would be. Thus, the objective of the following experiment was to determine the relative effects of 6 of the PG on food intake in rats.

Male rats (Charles River strain) were trained to bar press for pellets (Noyes; 100 pellets = 4.5 g) and were conditioned to one 2-h feeding per day. On experimental days, rats were injected s.c. with 1.0 ml/kg containing a dose of either 1 mg/ml of prostaglandins A_1, B_1, $F_{1\alpha}$, $F_{2\alpha}$, or 0.1 mg/ml of E_1 or E_2[12].

On control days, only the solvent was injected: 0.1 ml ethyl alcohol diluted with 0.9 ml of 0.02% Na_2CO_3 solution in saline (pH between 6.0 and 7.0). Immediately