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Summary

IAA-induced activation of purified acid phosphatase is reported and shown to determine an acceleration of phosphorolysis of starch.

Some Relationships Between the Pancreatic β-Cells and the Metabolism of the Epiphyseal Cartilage

I. *Cartilage ATP Concentration of Young Alloxan Diabetic Rats*

DE MOOR[1] and MARTINETTI, and ANDREANI[2] demonstrated that insulin deficiency provoked by alloxan diabetes produces, besides other biochemical and physiological alterations, a diminution of the body weight and of skeletal growth.

As the epiphyseal cartilage participates to the growth of the bone in length, and as in enchondral ossification the participation of the energetic phosphoric bonds is considerable (*i.e.* ATP, see CARTIER[3]), the author wishes to study whether the diminished growth observed in alloxan diabetic rats is accompanied in epiphyseal cartilage by a reduced concentration of ATP.

Albino male rats, Italicus strain, 50 days old, were divided into two groups. In the first group, after 12 h fasting, alloxan diabetes was provoked (Merck alloxan 200 mg/kg intraperitoneally); the second, which was the control group, was not treated. Ten days after alloxan treatment, and after 12 h fasting, blood sugar was measured according to NELSON[4], and in the diabetic rats (blood sugar 2·8–4·9 g/1000 cm³), analogously to those of the control group, body weight and blood sugar were controlled every 5th day for 20 days. Thirty days after the alloxan injection, having controlled body weight and blood sugar, all diabetic and normal rats, 12 h fasted, were anesthesized with Nembutal (5 mg/100 g body weight) and then killed by decapitation. The anterior and posterior limbs were removed as rapidly as possible, and placed in a beaker containing acetone and dry ice: the epiphyseal cartilages were weighed, homogenized with quartz sand and CCl_3COOH 10% at 0°C. After centrifugation, to the supernatant fluid, neutralized to pH 8·2, barium acetate was added. In the precipitate (barium insoluble fraction) ATP was measured after LE PAGE[5]. P was dosed according to the BEREMBLUM and CHAIN method[6]. It is necessary to emphasize the importance in such experiments of choosing rats of the same age: as a matter of fact, in a preliminary research, I observed that the ATP concentration of the epiphyseal cartilage shows, even for difference in their ages of 15–20 days, statistically significant variations. The decrease with age in ATP concentration in ossifiable cartilage is to be attributed to a progressive decrease during the growth of the intensity of the osteogenic power. Analogous observations, relative to the enchondral ossification, were made by ZAMBOTTI[7] on the behaviour of the cocarboxylase and β-glucuronidase, and by LORENZI on the behaviour of concentration of pyruvic acid[8].

The results obtained can be summarized as follows:

(1) The alloxan diabetic rats showed a remarkable diminution of the body weight with a check of growth (mean body weights at the start = 60 g; 30 days after alloxan treatment = 30 g. In the normal rats, the body weight increased from 60 g to 160 g in 30 days).

(2) The ATP concentration in epiphyseal cartilage of alloxan diabetic rats decreased by about 40% relative to normal rats (epiphyseal cartilage of normal rats = 20 mg P/ATP/100 g of wet tissue; epiphyseal cartilage of diabetic rats = 12 mg P/ATP/100 g of wet weight).

Such results clearly show that in alloxan diabetes the inhibition of skeletal growth is accompanied with a diminution of the ATP concentration in the epiphyseal cartilage.

To control whether the decreased ATP concentration in epiphyseal cartilage of alloxan diabetic rats can be attributed exclusively to the insulin deficiency, a group of albino male rats (of age and strain identical to those used in previous experiments) were treated with insulin (Zn-insulin protaminate 4 U.I./kg daily subcutaneously), ten days after alloxan treatment (blood glucose 2·8–4·8 g/1000 cm³). The results showed that in insulin-treated diabetic rats, hyperglycemia was much less intense than in untreated diabetic rats. In addition, 30 days after alloxan injection, the body weight was seen to be the same as that of the normal rats (*i.e.* 160 g) as was also the ATP concentration in epiphyseal cartilage (mg 20 P/ATP/100 g wet weight).

Therefore, in alloxan diabetic rats, both the decreased ATP concentration and the diminished osteogenic power of the epiphyseal cartilage, are to be referred exclusively to the insulin deficiency.

It may also be suggested that insulin plays an important role in regulating enchondral ossification, especially through its marked control of the multienzyme system which pertains in ATP metabolism and consequently in all biochemical reactions which are dependent on ATP (i.e. biosynthesis of glycogen from glucose; biosynthesis of cocarboxylase and chondroitin sulphate, etc.). However, this hypothesis requires further evidence.

These experimental results, besides explaining one of the mechanisms by which insulin influences enchondral ossification, are in good agreement with CARTIER's *in vitro* findings: the primary importance of ATP for normal behaviour in the osteogenic process[3].

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Riassunto

È stato studiato il contenuto di ATP nella cartilagine epifisaria di giovani ratti diabetici per allossana. Il deficit insulinico da diabete allossanico provoca, oltre ad arresto dell'accrescimento scheletrico, una diminuzione della concentrazione di ATP nella cartilagine di coniugazione. Il significato biologico di questo risultato è messo in relazione col meccanismo d'azione dell'insulina e con l'importanza dell'ATP nel processo di ossificazione encondrale.

Some Relationships Between the Pancreatic β-Cells and the Metabolism of the Epiphyseal Cartilage

II. *Cartilage Cocarboxylase Activity of Young Alloxan Diabetic Rats*

ZAMBOTTI[1] first discovered the presence of cocarboxylase in the epiphyseal cartilage. It was also established by ZAMBOTTI that this enzyme is strictly related to the intensity of the osteogenic process: in fact he showed that the cocarboxylase activity in the epiphyseal cartilage of young rabbits is inversely proportional to their age. I have reached identical conclusions in my researches in which the participation of cocarboxylase in various phases of evolution of the normal fracture callus[2] and also the behaviour of the cartilage cocarboxylase during growth in conditions (avitaminosis C) of reduced osteogenic power were studied[2]. These relationships, shown first by ZAMBOTTI, explain the histological and histochemical alterations of the epiphyseal cartilage demonstrated by ROASENDA and CAMURATTI[3] in deficiency of a particular vitamin which has the cocarboxylase as its active form, i.e. vitamin B_1.

I have studied the behaviour of the cocarboxylase in the epiphyseal cartilage of alloxan diabetic rats for the following reasons:

(1) Insulin deficiency is accompanied by an arrest of growth and alterations of osteogenic power of ossifiable cartilage[4].

(2) There is a strict relationship between the intensity of the osteogenic process and the amount of cocarboxylase activity[5].

(3) The diminution of the osteogenic power provoked by insulin deficiency is accompanied by a reduced concentration of ATP in the epiphyseal cartilage[6] in the alloxan diabetic rats. It is known that ATP is in large measure synthesized by means of the oxidation of two α-ketoacids (pyruvic and α-ketoglutaric)[7] for the oxidative utilization of which the cocarboxylase is essential.

(4) There is also a strict relationship between ATP and cocarboxylase regarding their metabolism. In fact, the biosynthesis of cocarboxylase from vitamin B_1 comes about by means of the reaction (B_1 + ATP→cocarboxylase + AMP)[8] catalyzed by the thiaminkinase, an endoergonic reaction which, since it receives the energy liberated by the demolition of ATP, should occur with minor intensity when the concentration of ATP is diminished.

Albino male rats, of Italicus strain, 50 days old, were divided into two groups. In one of these groups alloxan diabetes was provoked (200 mg of Merck alloxan, intraperitoneally). The control group was not treated. Ten days after the alloxan treatment, the body weight and blood sugar were controlled and then the diabetic rats (blood glucose after 12 h of fasting = 2·8–4·9 g/1000 cm^3) were controlled for another 20 days, with periodical examinations (control of body weight and of blood sugar every 5th day). 30 days after the alloxan treatment all the rats, diabetic and normal were killed, their body weight and blood sugar being controlled. The limbs were removed and placed in ice water. The cocarboxylase of the epiphyseal cartilages was extracted and measured according to the OCHOA and PETER's method[9], as modified by SILIPRANDI[10].

In the epiphyseal cartilages of the diabetic rats (which presented an arrest of skeletal growth identical to that previously described[6]) the cocarboxylase activity was reduced in respect to that in the control group (normal rats = γ 6·2/g wet weight; alloxan diabetic rats = γ 3·45/g wet weight). Results identical to those obtained in the normal rats were also demonstrated in diabetic rats treated with insulin (daily subcutaneous injection of Zn-insulin protaminate, 4 U.I./kg), from the 10th to the 13th day after the alloxan injection. It is therefore concluded that the cocarboxylase content decrease in the epiphyseal cartilage accompanying the arrest of the skeletal growth can be attributed in the alloxan diabetic rats to the insulin deficiency.

Coordinating my results on ATP and cocarboxylase with those obtained by other authors[11], it seems logical to deduce that a deficient production of insulin induces an arrest of osteogenesis provoking a reduced synthesis of ATP by damaging those reactions in which cocarboxylase is interested. On the other hand, the analogous behaviour of ATP and cocarboxylase in insulin deficiency leads us to suppose that in epiphyseal cartilage the metabolism of these two substance is interdependent: the insulin by means of the energy furnished by the ATP supports the transformation of thiamin into diphosphothiamin; the latter, in its turn, participates in the formation of ATP by means of the pyruvate and α-ketoglutarate oxidative decarboxylation. This hypothesis on the mechanism of the participation of the insulin in the osteogenic process by means of ATP and cocarboxylase is very probable, other than for my present results (osteogenesis normalization in the diabetic rats simultaneously with normalization of the amount of ATP and of cocarboxylase in the epiphyseal cartilage, after insulin treatment) also for my other experimental data: alloxan diabetes induces in the epiphyseal cartilage a diminished pyruvate and α-ketoglutarate oxidation; this process which is in strict relationship with the ATP synthesis[7], is also normalized by insulin treatment.

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