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Quantitative Studies on Enzyme Repression in the Developing Chick Embryo and Newly Hatched Chick.* (28006)

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An earlier report from this laboratory described the first known instance of negative feedback control of the level of a biosynthetic enzyme during embryonic development(1). The controlling compound is creatine, or a related metabolite, and the target enzyme is arginine-glycine transamidinase. This control has been operationally termed end-product repression, by analogy with bacterial systems, until its mechanism can be more completely elucidated(2). Since analogous feedback controls may be involved in the establishment and maintenance of tissue-specific enzyme patterns, closer study of the properties of the only known model system appears warranted. In this paper, quantitative and kinetic relationships between the controlling compound, and its precursors, and the target enzyme are examined.

Materials and methods. In general, the procedures employed were as described elsewhere(1). However, by modifying the physical state of creatine, more effective repression and more reproducible results can be obtained. Commercial crystalline creatine hydrate was dissolved in a minimal volume of water, with stirring, and an equal volume of acetone was added as quickly as possible. The suspension was allowed to settle, and the supernatant solution decanted. The residue was filtered with suction on a fritted glass funnel, washed thoroughly with acetone, spread on filter paper, and allowed to dry. The resulting fine powder suspends readily in water and is easily injected into eggs. Its large surface to volume ratio facilitates solution in the egg after injection. Transamidinase activities are reported as μmoles of hydroxyguanidine formed from arginine and hydroxylamine/hr/g liver (wet wt.).

Results. The compounds most effective in lowering the transamidinase level of embryonic chick liver are creatine, and its biosynthetic precursors. If creatine precursors must be converted to creatine before serving as a repressor, it might be expected that precursors of creatine metabolically proximal to the controlled reaction could not repress transamidinase completely, since the enzyme would be needed to form the repressor. Citrulline, 290 μmoles/egg injected on 7th day, represses transamidinase to 50% of control values by the 12th day. When glycine, 530 μmoles/egg, was injected with citrulline, transamidinase was repressed to 20% of control values. Almost identical results were obtained when arginine was substituted for citrulline. Apparently the chick embryo can convert citrulline to arginine, which then must react with glycine before the actual repressor is formed. In contrast to citrulline, ornithine does not repress transamidinase significantly. This is consistent with the fact that chicks lack ornithine transcarbamylase(3).

Fig. 1 shows dose-response curves for both creatine and guanidinoacetate. Efforts to modify the quantitative response to guanidinoacetate by simultaneously injecting potential competing methyl group acceptors have given inconclusive results, although repression can be antagonized significantly by compounds with similar charge distributions, such as betaine and 4-aminobutyrate. Efforts to achieve endogenous repression by encouraging hens to secrete repressors into the egg were unsuccessful: feeding laying hens for 20 days

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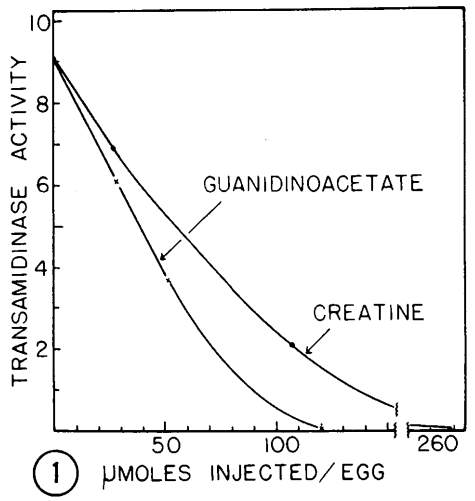

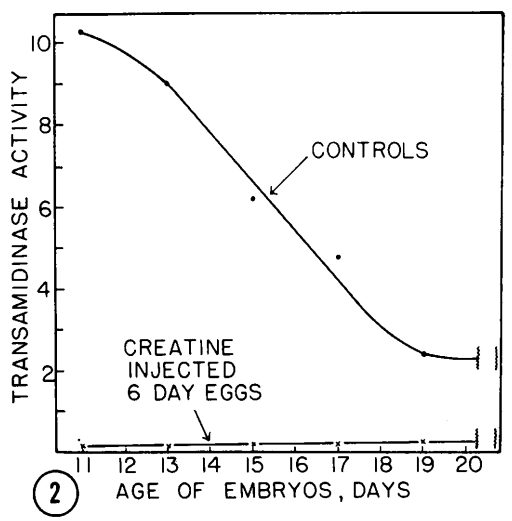

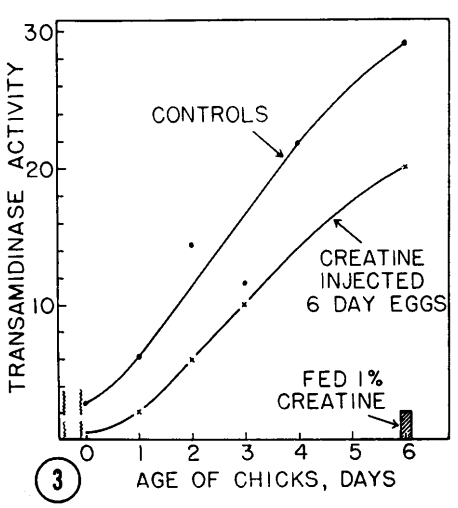

FIG. 1. Quantitative relationship between repressor concentration and extent of repression of transamidinase in chick embryo liver. Compounds injected into eggs on 7th day of incubation, and livers harvested on 13th day. Unlike creatine, guanidinoacetate is toxic to embryos at higher concentrations.

FIG. 2. Transamidinase activity of embryonic chick liver during development. Upper curve: normal pattern, showing characteristic decline in activity/g of liver prior to hatching. As result of liver growth during this period, total transamidinase/embryo does not decrease. Lower curve: injected creatine completely represses transamidinase throughout development.

FIG. 3. Continuation of experiment of Fig. 2, showing marked increase in transamidinase level in early post-hatch period, when chicks fed complete Startena diet. Creatine added to diet of controls prevents normal post-hatch increase in transamidinase/g liver.

a diet containing 2% creatine and 0.5% guanidinoacetate did not dramatically lower transamidinase levels of their 12-day embryos.

Fig. 2 demonstrates that essentially complete repression can be maintained during most, if not all, of development. Repression thus occurs in absence of intestinal flora, and prior to function of many endocrine glands. Even after being completely repressed, liver transamidinase increases in activity at the normal high rate after hatching (Fig. 3). Similar results were obtained when eggs were injected with creatine 1 day prior to start of incubation; increase in activity occurred following hatching, in the small fraction of embryos which survived injection of 0.3 ml of any liquid at that time. However, the increase in transamidinase activity occurs only when newly hatched chicks are fed a complete diet. When chicks are given no food, transamidinase activity declines to zero. When creatine is added to the complete diet, liver transamidinase stays at a low level, as indicated by the bar graph in Fig. 3.

Kinetic studies of the rate at which embryonic liver transamidinase is lowered following creatine injection were next undertaken. Such studies on tissues in the log phase of growth are important for an understanding of the mechanism of the creatine effect(2), particularly when compared to kinetic values obtained with non-growing, or slowly growing, tissues. Eggs which had been incubated for 8 days were divided into 2 groups. One group was injected with 270 μmoles of creatine on the 8th day, while the other group was in-

jected on the 9th day of incubation. Both groups were harvested and assayed on the 10th day. It was found that transamidinase was repressed to 43% of water-injected control values 1 day after creatine injection, and to 11% of control values 2 days after creatine administration.

Discussion. It is particularly significant for theories of differentiation that repression can be shown experimentally to occur during embryonic development. Heretofore it has been difficult to modulate enzyme levels prior to birth(4), although some success with hormonal induction has been reported(5). Monod and Jacob(6), using hypothetical circuits composed of known control elements, have described how interlocking, individually reversible, repressions might combine to give essentially irreversible differentiation. In their circuits, brief exposure to a single compound could determine subsequent enzyme patterns (*cf.* 7). During development, not only might individual gene function be modulated by cross repression or end-product repression, but whole groups of genes may be turned off or on at the proper time(8) by action of hormones, histones(9), or cross-reacting regulator systems(6).

Summary. Modification of the physical state of injected creatine has permitted a quantitative study of repression of arginine-glycine transamidinase by creatine, and its precursors, in liver of the developing chick embryo. Half-maximal repression was obtained by 65 μmoles/egg of creatine and 45 μmoles of guanidinoacetate. Complete repression occurred with 260 and 120 μmoles, respectively. Betaine and 4-aminobutyrate decrease the degree of repression attained. Arginine, and its precursor, citrulline, repress 50% at 290 μmoles/egg; when glycine is injected with either of these compounds, repression is increased to 80%. Ornithine, which cannot be converted to citrulline in the chick, does not repress. Laying hens fed creatine and guanidinoacetate have embryos with normal transamidinase levels. For the first time, essentially complete enzyme repression has been attained throughout embryonic development. However, derepression occurs during the 6 days following hatching, when chicks are fed a complete diet. In absence of food, or in presence of dietary creatine, this increase does not occur. The possible significance for theories of differentiation of the demonstration of enzyme repression during embryonic development is discussed.

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Effect of Cold Passage on Reproductive Capacity at Various Temperatures of Several Type 3 Poliovirus Strains.* (28007)

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The reproductive capacity of poliovirus strains varies depending upon the temperature of incubation during the replication cycle(1, 2). Strains vary, one from another, with regard to the temperature at which their repro-

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