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Histone Regulation of Lactic Dehydrogenase in Embryonic Chick Brain Tissue

Abstract. *Histone, when added at low concentrations to cultures of embryonic chick brain tissue, causes an inductive response in lactic dehydrogenase activity, whereas at higher concentrations of histone the response is repressive. This control is shown to operate by altering protein synthesis. Thus, histones exercise a primary regulatory function in the chick. Unlike lactic dehydrogenase, acetylcholine esterase is stable in this system and is not affected by histones or inhibitors of protein synthesis.*

Evidence of a cytological and biochemical nature suggests that histones take part in the regulation of genetic activity (*1*). The biochemical evidence shows that histones can inhibit the priming activity of DNA in directing RNA synthesis and that this inhibition is reversed when the histones are removed (*2*, *3*). Since polycationic molecules, such as polylysine, have a similar effect (*3*), the question arises whether histones have any specificity in inhibiting different regions of the DNA in a chromosome. Evidence for such specificity of histones on the chromatin from different tissues of the pea embryo has been presented by Bonner *et al.* (*4*). Since developmental processes involve both inductive and repressive responses, the position of histones as primary genetic regulators would be strengthened if it could be shown that under certain conditions they can bring about an induction or derepression of particular genes, while repressing these same genes under different conditions. We now present evidence that histones can indeed perform such an antithetic control function in the case of lactic dehydrogenase synthesis in embryonic chick brain tissue.

Small pieces (about 1 mm^3) of 14-day embryonic chick brain tissue (cerebral hemispheres) were cultured in Charity-Waymouth medium at 37°C in a CO_2-incubator on a gyratory shaker. Homogenates of washed tissue were assayed for lactic dehydrogenase (LDH) activity (*5*), and for acetylcholinesterase (AChE) (*6*). Protein was determined by the bromosulfalein method (*7*), and specific activities are expressed as micromoles of product (diphosphopyridine nucleotide or thiocholine, respectively) produced per hour per milligram of protein. Histone was prepared from the liver of 1-year-old White Leghorn chickens (*8*). The amino acid analysis showed a typical histone composition with a molar ratio of lysine to arginine of 1.97. For the measurement of amino acid–incorporating activity in the tissue ^{14}C-leucine was used. Radioactivity incorporated into protein, precipitated by 5 percent trichloroacetic acid from a 1*N* NaOH digest of the tissue, was measured on a Nuclear-Chicago counter.

The amount of lactic dehydrogenase in the control cultures was kept relatively constant by continuous synthesis and degradation (Fig. 1). Each point in this and subsequent figures represents an average value for three or more duplicate experiments. Actinomycin D (60 μg/ml) and puromycin (100 μg/ml) caused a decrease in enzyme activity which indicates a half-life for the enzyme of about 50 to 60 hours. The decrease of LDH activity in response to 100 μg of poly-L-lysine per milliliter (Fig. 1) is about the same as that caused by actinomycin. This repression by polylysine was not due to a direct inhibition of the enzyme, for no inhibitory effects were observed at the concentrations used.

In contrast to the effect (Fig. 1) of antibiotics and polylysine, which caused a decrease in the enzyme activity at all concentrations used (10 to 200 μg/ml), increasing the concentration of histone caused a stimulation of LDH synthesis which was maximum at 100 μg of histone per milliliter, but at a concentration of 400 μg/ml became

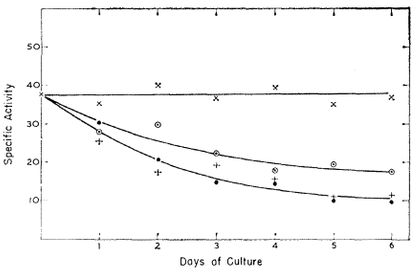

Fig. 1. Lactic dehydrogenase activity in cultures exposed to 60 μg/ml of actinomycin D (● ——— ●), 100 μg/ml of puromycin (⊙ ——— ⊙), and 100 μg/ml of poly-L-lysine (+ ——— +), compared with the control (× ——— ×).

a repression comparable to that caused by antibiotics or polylysine. Commercial calf thymus histone caused the same pattern of response, although the magnitude was slightly different (Fig. 2, upper curve). As with polylysine, the effect of 400 μg of histone per milliliter was not due to a direct inhibition of the enzyme, since there was no such inhibition in incubated homogenates until relatively high concentrations of histone were reached (5 to 10 mg/ml). A neutral protein such as bovine serum albumin at concentrations from 10 to 1000 μg/ml had no effect upon LDH activity in the cultures.

Evidence that the observed increases and decreases of LDH activity were not due to the accumulation of dissociable activators or inhibitors was provided by the fact that mixing and dilution experiments on homogenates

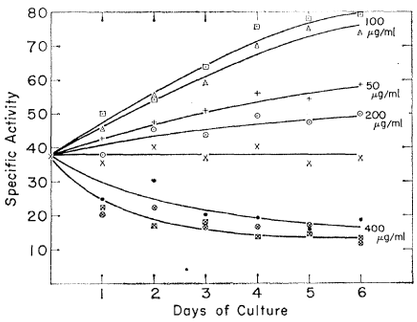

Fig. 2. Lactic dehydrogenase activity in cultures exposed to the concentrations of adult chicken liver histone shown in curves △ ——— △, + ——— +, ⊙ ——— ⊙, and ● ——— ● compared with the control (× ——— ×). Upper curve (⊡ ——— ⊡) is the response to 100 μg/ml of commercial calf thymus histone. Lower curve: 100 μg/ml of chicken liver histone plus 60 μg/ml of actinomycin D (⊠ ——— ⊠) and 100 μg/ml of chicken liver histone plus 100 μg/ml of puromycin (⊗ ——— ⊗).

of high and low specific activity always gave the predicted results. The two lowest curves of Fig. 2 indicate, further, that the observed responses to histone were due to changes in the ability of the tissue to synthesize protein, since the inductive effect of chicken liver histone (100 μg/ml) on LDH activity was abolished by either actinomycin D (60 μg/ml) or by puromycin (100 μg/ml). Comparison of Figs. 1 and 2 shows that the decrease of LDH activity was at least as great when the concentration of histone was 100 μg/ml together with puromycin or actinomycin as when the antibiotics acted alone. This indicates that the effect of histone could not be attributed to an increased half-life of the LDH molecules rather than to an enhanced rate of synthesis; if this had been the case one would expect that the decrease of the enzyme activity in response to puromycin or actinomycin D plus histone would be at a slower rate than that in the absence of histone.

The difference between the effect of histones and that of these metabolic inhibitors is brought out in Fig. 3. Histone or inhibitor was added to the cultures when they were prepared, and the culture medium was not changed thereafter. The ^{14}C-leucine–incorporating activity of the tissue was determined during a 2-hour incorporating period, by which time the controls were characterized by about 20,000 count/min per milligram of protein. The results are presented as percentages of the control.

Puromycin, actinomycin D, and polylysine arrested the amino acid–incorporating activity of the tissue within 24 hours or less. Puromycin was effective in blocking protein synthesis within 5 minutes of its addition to the culture. In contrast, histone (calf thymus or from chicken liver) can enhance or reduce the amino acid–incorporating activity of the tissue, depending upon the concentration used. At a concentration of 100 μg/ml histone produces maximum induction of LDH and causes an increase in incorporating activity, whereas at 400 μg/ml repression occurs and incorporating activity is reduced to 70 percent of the control. It may be significant that the repression of LDH caused by 400 μg of chicken liver histone per milliliter (Fig. 2) is not greatly different from that produced by puromycin, actinomycin D, or poly-L-lysine (Fig. 1), whereas this same concentration of histone reduces the overall protein-synthetic activity of the tissue much less than the metabolic inhibitors. This suggests that the histone is exercising a degree of selective repression on protein synthesis, some proteins being affected much more than others, unlike the uniform and complete suppression of protein synthesis produced by the antibiotics and by polylysine.

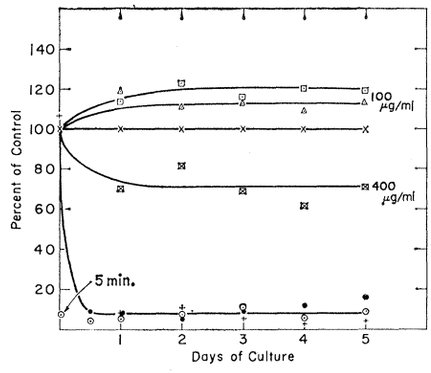

Fig. 3. Amino acid (^{14}C-leucine)–incorporating activity of cultures exposed to: chicken liver histone at concentrations of 100 μg/ml (△ ——— △) and 400 μg/ml (⊠ ——— ⊠), 100 μg/ml of calf thymus histone (⊡ ——— ⊡), 60 μg/ml of actinomycin D (● ——— ●), 100 μg/ml of puromycin (⊙ ——— ⊙), and 100 μg/ml of poly-L-lysine (+ ——— +), compared with the control (× ——— ×).

If the rate of the decrease in synthesis of protein by embryonic brain tissue represents the decay of messenger RNA in embryonic brain cells, then the mean half-life of the messenger molecules in this tissue is about 70 minutes. This was demonstrated in an experiment which measured the decrease of ^{14}C-leucine–incorporating activity of tissue cultures over a period of 12 hours after exposure to 60 μg of actinomycin D. From this experiment it is evident that embryonic brain cells have a very active nucleic acid metabolism (*9*).

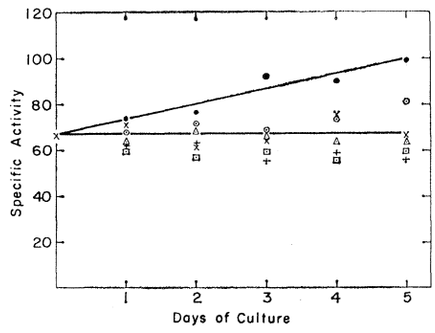

Fig. 4. Acetylcholinesterase activity in cultures exposed to 100 μg/ml of chicken liver histone (△ ——— △), 100 μg/ml of calf thymus histone (⊡ ——— ⊡), 100 μg/ml of poly-L-lysine (+ ——— +), 100 μg/ml of puromycin (⊙ ——— ⊙), and 60 μg/ml of actinomycin D (● ——— ●), compared with the control (× ——— ×).

The specific activity of lactic dehydrogenase was virtually unchanged during the period of chick brain development from 13 days to 18 days of incubation (37.5 μmole/mg of protein per hour at 13 days, 40 μmole/mg of protein per hour at 18 days). This enzyme plays a role in the general metabolic activity of cells, and occurs in all tissues of the chick embryo. In contrast, the enzyme acetylcholinesterase is quite specific to brain and certain other tissues; its specific activity in the cerebral hemispheres changes from 47.5 μmole/mg of protein per hour at 13 days of incubation to 140 μmole/mg of protein per hour at 18 days. Comparison of the behavior of AChE with that of LDH in response to histones, polylysine, puromycin, and actinomycin D (Fig. 4) reveals that the control remained unchanged, apart from variations during the 5 days of culture, unlike the tissue in the embryo. However, this steady concentration of AChE was not maintained by constant synthesis and degradation of the enzyme, as is the case with LDH. The amount of enzyme was stable to puromycin and polylysine, which were shown to arrest protein synthesis; actinomycin D produces an anomalous increase in specific activity (*10*). Furthermore, there was no significant change in AChE activity upon the addition of chicken liver or calf histone to the cultures at any concentration used. It would appear, then, that the synthesis of this enzyme stops under tissue culture conditions, and that the enzyme molecules which were present at the commencement of tissue culture (14-day brain) are stable during the culture period. The anomalous increase in enzyme activity with actinomycin is unexplained. What makes AChE stable in these cells in contrast to LDH, which is constantly turning over, is a question of some interest in relation to mechanisms of cellular differentiation, but the present study can cast no light on this problem.

The evidence suggests that the responses to histones were due to changes in the control of messenger synthesis by the DNA in the embryonic brain cells, although the observations are also consistent with an explanation invoking the idea of histone control at

the level of the messenger molecules themselves (*11*). Not excluded at this time is an explanation based on the toxic and irritant action of histones on intact, living cells (*12*); many poisons act as stimulants at low concentrations and become inhibitory only at higher dosages. A more plausible interpretation of the results presented, however, is that an inducer acts by inactivating a repressor, and that the primary mechanism of control in cells is always repression. This idea is parallel to the induction-repression hypothesis of genetic regulation (*13*). Our observations can then be explained if it is assumed that the different species of histone molecule present in the total histone extract of chicken liver or calf thymus nuclei have different affinities for different parts of the DNA. The molecular heterogeneity of total histone extracts has been shown by Neelin and Butler (*14*), who obtained up to 18 bands by starch-gel electrophoresis of histones from chicken spleen, liver, erythrocytes, heart, and testis. There is no direct evidence that these fractions have differential affinities for different parts of the DNA, but this specificity seems quite plausible. Even actinomycin D appears to have a differential affinity for different genetic loci in *Escherichia coli*, depending presumably upon the incidence of guanine bases (*15*).

Since the primary effect of histones on DNA is an inhibition of its priming activity, the observed inductive response of LDH to low concentrations of histone may be explained as a secondary result due to the repression of another genetic locus which directs the synthesis of either an aporepressor or a corepressor of the LDH gene, or rather genes, since isozymes of this enzyme occur in chick brain (*16*). This requires that the histones have a higher affinity for this locus than for the LDH genes, so that it is most affected when there is little histone present. As the histone concentration is increased, repression will extend to more loci, with the result that at some concentration the LDH genes will themselves experience a repression. The fact that the amino acid–incorporating activity of the brain tissue can also be increased by relatively low concentrations of exogenous histone indicates that these histones first repress some key loci whose activity tends to control not only LDH synthesis but also the general degree of protein synthesis of the cell. These may be the regulator genes as a group, or they may be some loci directing the synthesis of a set of corepressors with multiple sites of action on the DNA. The added chicken liver or calf thymus histone must then have a relatively high affinity for these key loci so that they are first selected for repression, thereby releasing other loci and raising the amount of general protein synthesis in the cells.

If a step further is taken and histones are identified with aporepressors, then there is the interesting possibility that histones could control their own synthesis in cells. The general picture would thus be that histones are partitioned in their repressive function between histone-producing loci on the DNA (the regulator genes) and other sites directing nonhistone protein synthesis (the operator genes). At different histone concentrations within a cell, structural genes will be differentially influenced, whether derepressed or repressed as in the case of LDH, and at the same time the rate of histone synthesis will be regulated by the amount of histone itself. In this manner the problem of regulating the controls can be resolved by closing the causal sequence of regulation in the cell.

Thus, the synthesis of a particular protein, lactic dehydrogenase, can be almost completely shut off with a concentration of histone (400 μg/ml) which reduces general protein synthesis to only about 70 percent of the control. This indicates that concentration of histone is an important variable in the selection of cellular states (some genes off, some on at different levels of activity). It is then not necessary to have a large number of distinct histone species in order to generate many stable cellular states, since differing relative concentrations of a few histone species would be effective in producing the different states. This could explain why it is that investigators have often failed to find obvious tissue-specificity of histones (*17*), since the specificity could reside in concentration rather than in type of histone present. These considerations offer another basis for suggesting that histones may be primary regulators of genetic activity, and that they may be identified with the postulated but as yet unidentified aporepressors.

B. C. GOODWIN*
I. W. SIZER

Department of Biology, Massachusetts Institute of Technology, Cambridge 02139

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* Present address: Institute of Edinburgh, Edinburgh, Scotland.

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Cerebellar Disease in Cats Induced by Inoculation of Rat Virus

Abstract. *Rat virus selectively destroys the external germinal layer of the cerebellar cortex when inoculated intracerebrally into newborn cats. The lesions are marked by numerous intranuclear inclusion bodies and a rise of virus titers. These effects suggest that the spontaneous ataxia of cats which is accompanied by cerebellar hypoplasia, may be of viral origin.*

In a previous report (*1*) we described a lesion of suckling hamsters in which rat virus (*2*) selectively destroys the external germinal layer of the cerebellar cortex and thus induces hypoplasia of the cerebellum and chronic ataxia. Our finding that a similar condition in domestic cats had been described in 1888 (*3*) prompted us to search for a virus as the causative agent of feline ataxia and to study the effects of various strains of rat virus on cerebellar ontogenesis following intracerebral inoculation of neonatal cats.