## Tissue Specificity of Histone Phosphorylation

Abstract. *The incorporation of $^{32}P$-phosphate into histone fractions isolated from normal, hepatectomized, and Novikoff hepatoma-bearing rats was investigated. Varying degrees of phosphorylation were exhibited by different histone fractions. The phosphorylation of histones is tissue specific and appears to be correlated with metabolic cell functions, that is, it decreases with increasing mitotic activity of the cells.*

The gene regulatory function ascribed to histones was originally explained by attributing tissue and species specificity to histones (*1*). However, improved methods utilized for the analysis of histones have failed to demonstrate a significant degree of species and cell specificity (*2*). Recently, new concepts for interpreting genetic regulation by histones have emerged. Current investigations have suggested that the derepression by histones may be affected by chemical modification of the histones. Acetylation (*3*), methylation (*4*), and phosphorylation (*5, 6*) of histones are among the possible mechanisms for decreasing their repressor activity.

Histone phosphorylation has been demonstrated to be energy dependent and occurs after the histone has been completely biosynthesized (*6, 7*). An enzyme which specifically phosphorylates the seryl residues of histones, as well as protamines, has been isolated from liver (*8*). This histone phosphokinase, although capable of phosphorylating all histone fractions, exhibited a marked preference for the very lysine-rich histone F1 (*8*).

In an effort to determine whether this alteration indeed reflects changes in the template activity of chromatin, we initiated a study of the phosphorylation of histones under different growth conditions. Male albino rats (200 to 250 g) were subjected to subtotal hepatectomy under ether anesthesia (*9*), and the livers were allowed to regenerate for 36 hours. Novikoff hepatoma was transplanted intraperitoneally, and the animals received an isotope injection 6 days after the transplantation. Normal, hepatectomized, and Novikoff hepatoma-bearing rats that had been injected intraperitoneally with either 1.5 mc of $Na_3{}^{32}PO_4$ (carrier free) per kilogram or 20 $\mu$c of $^{14}$C-l-lysine per kilogram (240 mc/mmole) were killed 1 hour after injection, and livers, spleens, and ascitic fluid were removed.

Purified nuclei from the different tissues were washed with 0.14*M* NaCl containing 0.01*M* trisodium citrate, with 0.1*M* tris buffer, *p*H 7.6, and with 95 percent ethanol (*10, 11*). The nucleohistone was subsequently extracted with a mixture of ethanol and 1.25*N* HCl (4 : 1 vol/vol) to obtain the arginine-rich histones F2a and F3. Extraction of the ethanol-HCl residue with 0.2*N* HCl yielded the lysine-rich F1 and F2b histones. After precipitation with acetone the histones were dialyzed and lyophilized.

The isolated F1F2b (lysine-rich) and F2aF3 (arginine-rich) fractions were further fractionated by quantitative starch gel electrophoresis (*11*). Half of the eight gel strips which were run parallel were cut, and the sliced bands were either suspended in counting fluid to be counted directly or were burned in oxygen and subsequently counted in a liquid scintillation spectrometer (*12*). The remaining four gels were used for determining the protein concentration of the corresponding bands. The specific activities (counts per minute per milligram of protein) of $^{32}$P and $^{14}$C were computed from the average values of each set of four gel strips. The relative activities ($^{32}$P relative to $^{14}$C) of the different histone fractions obtained from two experiments are reported in Fig. 1. The designations of the histone fractions (F1, very lysine-rich; F2b, moderately lysine-rich; F2a and F3, arginine-rich) refer to the electrophoretic bands located in different positions. These bands have been positively identified by comparison of electrophoretic patterns of known histone fractions, as well as by their composition which has been determined for these four fractions. The "F3" fraction extracted with F1F2b has a slow mobility comparable to that of F3 but probably is not F3. Since its composition has not been established, it is uncertain whether $^{32}$P is truly incorporated into histone or whether the radioactivity is due to the presence of nucleic acids. However, the possibility that the radioactivity of this histone fraction, as well as of others, could be attributed to nucleic acids was virtually eliminated by the results obtained from a double-labeled experiment performed with $^{3}$H-cytidine and $^{3}$H-thymidine with $Na_3{}^{32}PO_4$. In this experiment rats received an injection of 0.5 mc of $^{3}$H-cytidine per kilogram (specific activity, 6.0 c/mmole) and 0.5 mc of $^{3}$H-thymidine per kilogram (specific activity, 15.0 c/mmole) in addition to the $Na_3{}^{32}PO_4$. Since none of the fractions isolated from these rats contained significant amounts of the tritiated compounds, it was assumed that the radioactivity represented true incorporation of $^{32}$P into histone fractions.

Although the data indicate that all histone fractions are capable of accepting the $^{32}$P-phosphate, the extent of phosphorylation is significantly higher in certain fractions. Furthermore, comparison of the relative ($^{32}$P relative to $^{14}$C) specific activities of histones in liver, regenerating liver, spleen, and Novikoff hepatoma strongly suggests that the phosphorylation of histones

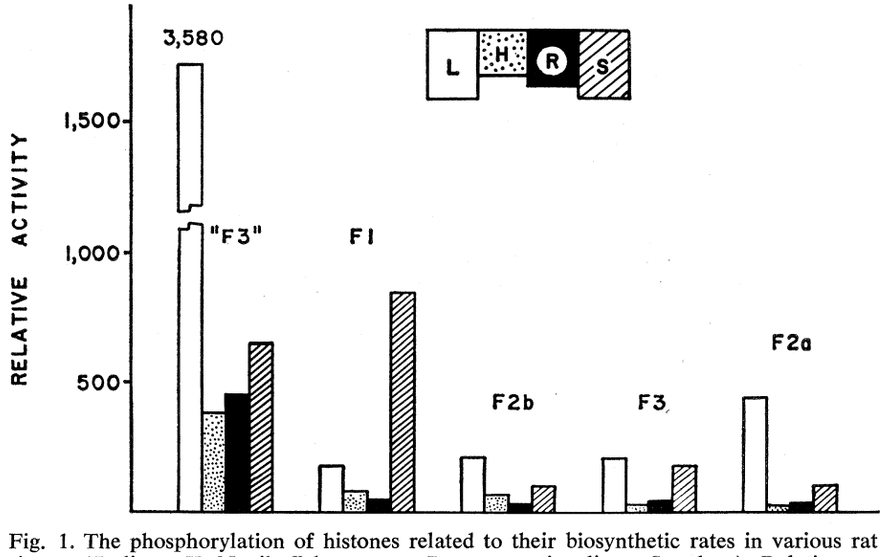


Fig. 1. The phosphorylation of histones related to their biosynthetic rates in various rat tissues (*L*, liver; *H*, Novikoff hepatoma; *R*, regenerating liver; *S*, spleen). Relative activity is expressed as [(cpm $^{32}$P/mg histone)/(cpm $^{14}$C/mg histone)] $\times$ 100.

is tissue specific. Well-differentiated tissues, such as liver or spleen, turn over their histone-bound $^{32}P$ faster than the similar fractions in regenerating liver or in hepatoma where a less-differentiated environment is found. The uncertainty of when maximum incorporation of $^{32}P$ occurs in regenerating liver imposes certain limitations. A twofold increase in the level of phosphate in the F1 fraction has been shown to occur 16 to 24 hours after partial hepatectomy (*13*). Since DNA and histone biosynthesis exhibit two peaks, one occurring about 20 hours and the other approximately 28 to 43 hours after hepatectomy (*14*), it was assumed that 36 hours of regeneration selected for these experiments were most likely in the range of maximum phosphorylation of histones.

Also, it appears that the active phosphorylation of histones is associated with metabolic functions of the cells and not with the growth, since the relative extent of $^{32}P$ incorporation decreases with increasing mitotic rates. More histone is synthesized by regenerating liver or hepatoma cells than by normal liver, but a greater amount of histone is phosphorylated in liver than in other tissues. Variability with age was observed; very young rats exhibited a marked increase (approximately twofold) in phosphorylation.

Ord and Stocken (*13*) demonstrated the sensitivity of changes in phosphorylation to irradiation and partial hepatectomy. Irradiation significantly depressed the incorporation of $^{32}P$ into the F1 fraction by thymus and regenerating rat liver; on the other hand, nonirradiated regenerating livers showed a twofold increase in $^{32}P$ incorporation 16 to 24 hours after partial hepatectomy. Another study by Kleinsmith and co-workers revealed that in isolated rat thymus nuclei (*6*) the phosphoserine content of the F1 fraction is three times greater than that of the F3 fraction. The results reported here tend to support the concept that histone phosphorylation may function in genetic derepression, as was suggested by Allfrey *et al.* (*15*).

Further evidence supporting the presence of phosphate in histones as phosphoserine was recently obtained. Isolated tryptic peptides of histones which have been fractionated by gel filtration on Sephadex G-75 were analyzed after partial hydrolysis with the aid of an automatic amino acid analyzer, and ninhydrin-positive, $^{32}P$-containing peaks were identified by means of a scintillation flow detector. The results indicate that serine-O-phosphate, the first peak to appear during the analysis, was responsible for the $^{32}P$ activity in the peptides.

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## Lysergic Acid Diethylamide: Effect on Embryos

Abstract. *Injection of lysergic acid diethylamide tartrate into mice in early pregnancy caused a 57 percent incidence of grossly abnormal embryos.*

A variety of agents, such as x-irradiation, viruses, drugs, vitamins, hormones, and hypoxia, are potent inducers of developmental malformations when administered to pregnant mice. Generally, the type and extent of abnormalities that may be produced depend on the stage of development reached at the time of experimental insult as well as on the nature and concentration of the agent used (*1*). These studies represent extension of an individual student project originally carried out as part of a class in developmental biology. The results of this project (experiment 1, Table 1) were sufficiently provocative to warrant more systematic examination.

Mice of strains BALB/CAu, C57BL6/Au, C3H/HeAu, and $F_1$ (BALB/C × 57BL) were used. Mice were checked for vaginal plugs daily, the day of appearance of the plug being taken as day 0 of development. Lysergic acid diethylamide (LSD-25) was obtained from the National Institutes of Health as 0.1 mg of lysergic acid diethylamide tartrate (Sandoz, batch No. 65002) per milliliter. Before injection, the LSD was diluted in Ty-

Table 1. Embryonic abnormalities induced by LSD-25 injected into mice on day 7 of pregnancy.

| Experiment | LSD-25 (g) | Litters (No.) | Embryos (No.) | Normal (No.) | Abnormal (No.) |
|---|---|---|---|---|---|
| | | *C3H* | | | |
| 1 | $5 \times 10^{-7}$ | 4 | 29 | 6 | 23 |
| | $5 \times 10^{-8}$ | 6 | 43 | 14 | 29 |
| 2 | $2 \times 10^{-7}$ | 3 | 29 | 16 | 13 |
| 3 | $1 \times 10^{-6}$ | 1 | 10 | 5 | 5 |
| | | *C × C57BL* | | | |
| 2 | $5 \times 10^{-7}$ | 1 | 8 | 5 | 3 |
| | $3 \times 10^{-7}$ | 1 | 8 | 5 | 3 |
| | $1 \times 10^{-7}$ | 1 | 9 | 9 | 0 |
| | | *C57BL* | | | |
| 3 | $1 \times 10^{-6}$ | 1 | 7 | 1 | 6 |
| | | *BALB/C* | | | |
| 2 | $5 \times 10^{-7}$ | 2 | 15 | 6 | 9 |
| | | *Control mice* | | | |
| 2* | | 4 | 31 | 28 | 3 |
| 3† | | 5 | 33 | 31 | 2 |

* Tyrode's solution injected as control. † Sodium potassium tartrate injected as control (see text).