Zeitschrift: Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche

Herausgeber: Schweizerische Akademie der Medizinischen Wissenschaften

Band: 24 (1968)

Artikel: The uptake of histones by mammalian cells

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DOI: https://doi.org/10.5169/seals-307738

D.C.: 612.014.1

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The Uptake of Histones by Mammalian Cells

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Histones are basic proteins which are bound ionically to the DNA in the chromatin of all nucleated cells. This close association with DNA has led to many speculations that histones may function in the control of genetic expression [8]. However, there is as yet no experimental evidence which supports conclusively such a model.

In preliminary studies of possible effects of histones on gene expression, measured by specific enzyme levels, in mammalian cells in tissue culture, we investigated if histones can be taken up into the cells and in which part of the cell they are bound. These studies have been published [4], and are described briefly here. Histones were labelled with fluorescein (by the procedures routinely used for labelling antibodies) and added to monolayer cultures of L cells. Uptake into the cells was observed qualitatively using the fluorescence microscope, and also quantitatively by extraction of the labelled histone and measurement of fluorescence.

Uptake of labelled histones. – After short periods (15 min) labelled histones are bound predominantly on the cell surface. Later, after 1 hour, they are found in a granular distribution in the cytoplasm (Fig. 1). Each cell takes up between 1 and 2×10^6 molecules of histone when the medium contains $10\,\mu g$ of histone per ml (Fig. 2). Even after very long periods (up to 24 hours), however, labelled histones could never be detected in the cell nucleus, even when frozen sections were examined to eliminate fluorescence in the layer of cytoplasm overlying the nucleus. It thus appears that histones from outside the cell are not able to penetrate the nucleus where they could affect the activity of nuclear genes. Rather, those molecules which enter the cell are bound in the cytoplasm, probably to RNA [2].

Effects of histones on genetic activity. – Several reports [1, 3] have been published describing effects of histones on gene function in mammalian cells. However, these results must be evaluated with great caution because of the marked toxic effects of histones on mammalian cells, effects which are probable due to destruction of the cell membrane [5] and which may thus lead to inactivation or loss of cellular enzymes. These toxic effects can be observed at concentrations as low as $30\,\mu g$/ml [5], whereas effects on levels of enzyme activity are observed only at concentrations of $100\,\mu g$/ml and higher [1, 3].

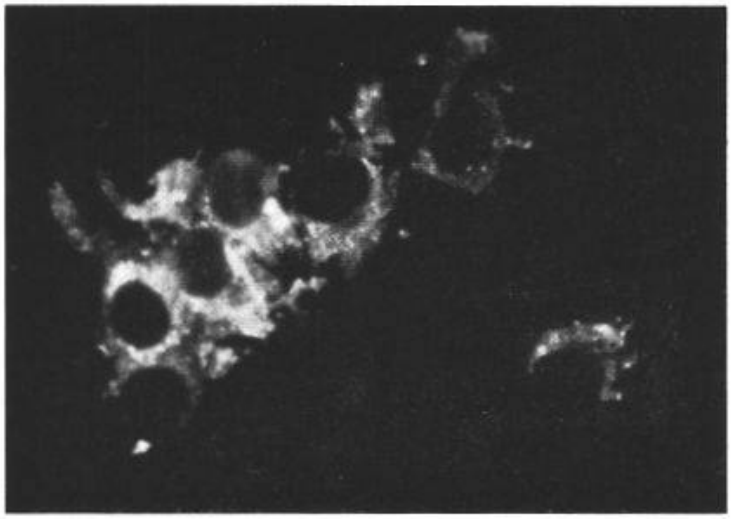

Fig. 1. The pattern of distribution of fluorescein-labelled histones in L cells, incubated for 2 hours with histone at 30 μg/ml.

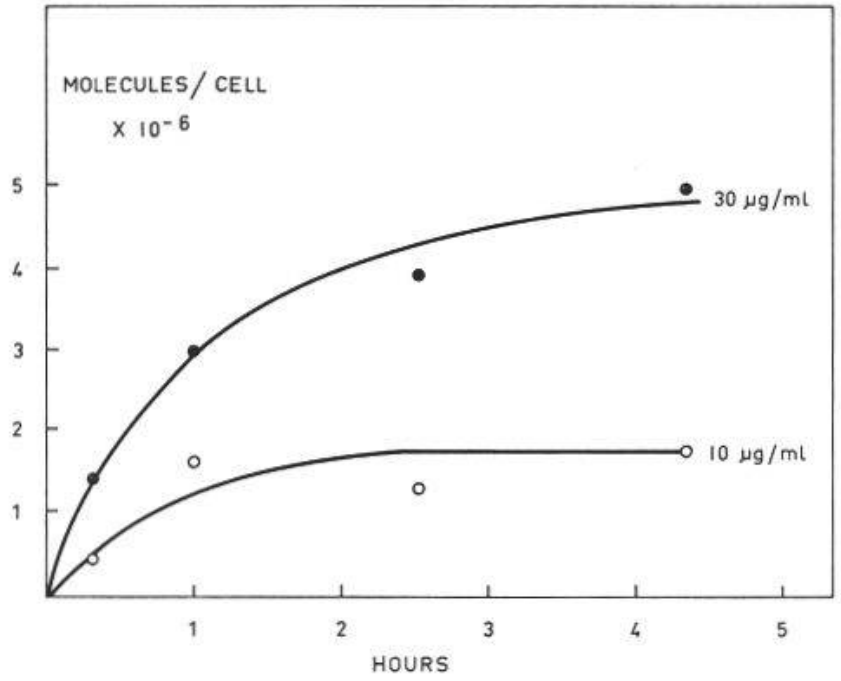

Fig. 2. Kinetics of uptake of fluorescein-labelled histone by L cells at histone concentrations of 10 and 30 μg/ml. The cells were dissolved in sodium dodecyl sulphate (0.5%), and fluorescence measured in a Farrand fluorimeter.

From our observations that extracellular histones are apparently not able to reach the nucleus, and because of their toxic side-effects, we conclude that meaningful studies of histone function can only be made using subcellular systems such as purified chromatin [6, 7].

Summary

Histones were labelled with fluorescein and their uptake into cells in tissue culture was studied. The labelled histone molecules attach first to the

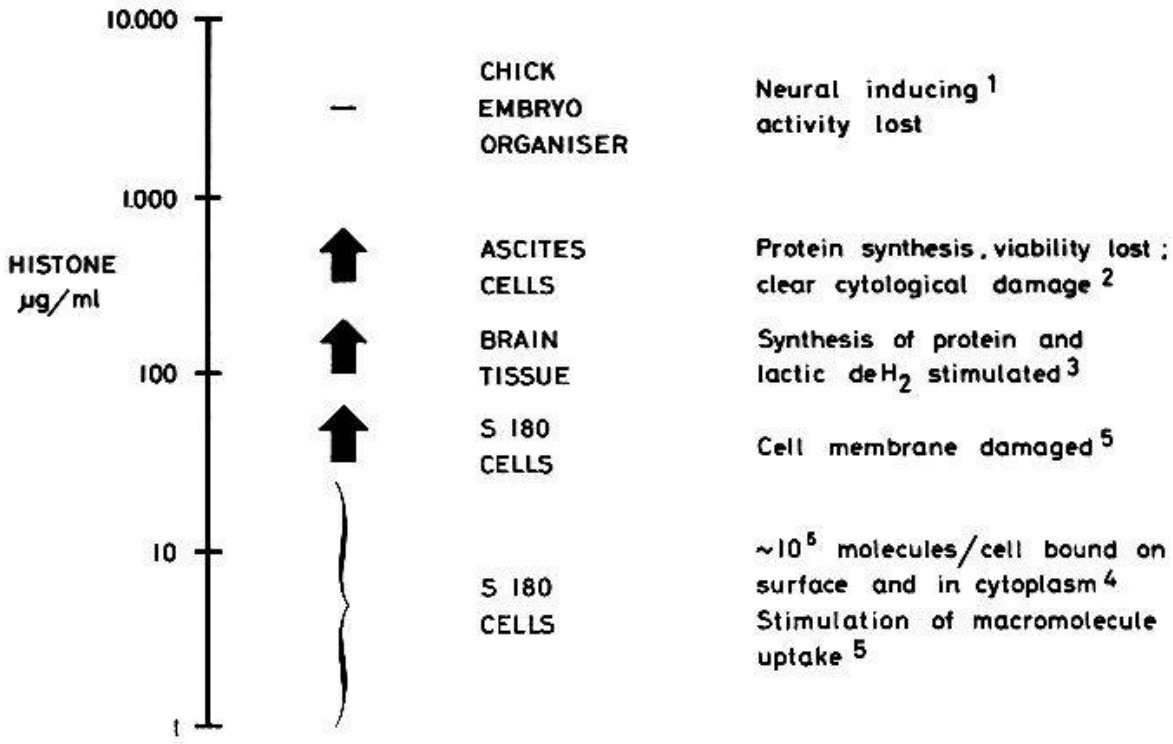

Fig. 3. A summary of some effects of histones on cells and tissues *in vitro*, and the concentration at which these effects are induced.

cell membrane, and later reach the cytoplasm, but are never found in the nucleus. These results make it unlikely that extracellular histones could affect the activity of nuclear genes. Although other reports have claimed such an action, the use of histones at concentrations which damage the cell membrane throws doubt on this interpretation of these experiments.

Zusammenfassung

Histone wurden mit Fluoreszein markiert, um ihre Aufnahme in die Zellen von Gewebekulturen zu studieren. Die markierten Histonmoleküle halten sich zuerst an der Zellmembran fest und erreichen später das Zytoplasma, werden aber nie im Zellkern aufgefunden. Diese Resultate lassen es als wenig wahrscheinlich erachten, daß extrazelluläre Histone die Tätigkeit der Gene in den Nuklei beeinflussen könnten. Obwohl in anderen Berichten eine solche These aufgestellt wird, so läßt doch die Anwendung von Histonen in Konzentrationen, welche die Zellmembran schädigen, an dieser Auslegung der Experimente zweifeln.

Résumé

Après avoir marqué des histones avec de la fluorescéine, l'on étudie leur incorporation dans les cellules de cultures de tissus. Ces molécules d'histones marquées se fixent d'abord sur la membrane cellulaire, puis atteignent le cytoplasme, mais n'apparaissent jamais dans le noyau de la cellule. Ces résultats rendent improbable une influence des histones extracellulaires sur l'activité des gènes dans le noyau. Bien qu'il y ait eu des travaux proposant une telle action, l'application d'histones à des concentrations attaquant la membrane cellulaire nous fait douter de cette interprétation de ces expériences.

Riassunto

Per studiare la loro incorporazione nelle cellule delle colture di tessuti, gli istoni furono marcati con fluorescina. Le molecole marcate degli istoni aderiscono dapprima alla membrana cellulare e raggiungono più tardi il citoplasma ma non si trovano mai nel nucleo della cellula. Questi risultati ci fanno concludere che sembra poco probabile che gli istoni extracellulari possano influenzare l'attività dei geni nei nuclei. Quantunque in altre comunicazioni si sostenga una tale tesi, l'uso degli istoni in concentrazioni atte a danneggiare la membrana cellulare, fa dubitare che l'interpretazione di tali esperimenti sia corretta.

Acknowledgements. – This work was carried out during tenure of a Fellowship of the Jane Coffin Childs Memorial Fund, and owes much to the encouragement and advice of Dr Harold Amos, Harvard Medical School, Boston, USA.

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