Histone Acetylation in Scrapie-Affected Mouse Brain

Scrapie is a degenerative condition of the central nervous system occurring spontaneously in sheep though it has in recent years been transferred experimentally to small rodents[1,2]. The most striking structural change found in scrapie is the marked hypertrophy of the astrocytes first considered as the primary site of damage by FIELD[3] and PATTISON[4]. This observation of the occurrence of 'activated' cells suggested that examination of factors believed to control chromosomal function might provide additional information on the function of the elusive scrapie agent. ALLFREY et al.[5] showed that acetylated histones, though still strongly bound to DNA, are only poor inhibitors of RNA synthesis. Further, lymphoid cells activated with phytohemagglutinin were shown to produce an excess of acetylated histone and that this process preceded the formation of new RNA and DNA[6], it then follows that a disturbance in histone acetylation resulting from the action of an agent, e.g. virus, may produce specific cellular activation. The present work describes an in vivo study of histone acetylation in the central nervous system of normal and scrapie mice.

Materials and methods. Mice. Scrapie was induced in BSVS mice by i.c. inoculation of 0.05 ml of a 1 in 10 scrapie mouse brain suspension centrifuged at 1500 *g* for 10 min. Clinical diagnosis was made as described by CHANDLER[7] and a small piece of mid-brain was taken for histological examination at sacrifice.

Tracer techniques. Mice were injected i.c. with 2.5 μC of sodium acetate-2-C^{14} taking great care to avoid leak-back. The animals were killed by exsanguination under anaesthesia and the brains removed rapidly for extraction of histones. Radioactivity was measured in a Packard Tricarb liquid scintillation counter using dioxan scintillator giving an efficiency of 60% for C^{14}. As all measurements were made under strictly comparable conditions no correction was made for quenching.

Extraction of histone. Mouse brains were removed rapidly and cooled to 0 °C. The brains were weighed, homogenized with 3 times their volume of 15% (w/v) sucrose containing 2 m*M* $CaCl_2$ and the resulting suspension centrifuged at 1600 *g* for 15 min at 4 °C[8]. The final nucleoprotein pellet was washed with cold 88% ethanol-0.01*N* HCl to remove a comtaminant[6] and the washed sediment then extracted twice with 0.2*N* HCl for 20 min to obtain the histones. Extracts were combined and histones precipitated with acetone, washed with acetone, ether and finally air dried. No further purification of the product was attempted and it was termed 'histone' although probably contaminated with some non-specific basic proteins.

Analytical methods. Protein concentration was estimated in duplicate using the method of LOWRY et al.[9].

Results. The time course of acetate incorporation into the histone fraction is shown in Table I. The reaction is essentially complete after 2 h in distinction from the incorporation of lysine into brain histones (CASPARY, unpublished). At this stage over 95% of radioactivity was precipitable with trichloroacetic acid.

The acetylation of histone in brain was studied in groups of 4 animals with controls matched for age and sex. The Figure shows the appearance of significantly increased acetylation (> 2 S.D.) in the histone fraction only 3 months following the inoculation of scrapie brain. This increase is of the order of 25% and remains constant after its first appearance. The mice in the 3-month group had neither clinical symptoms nor did histology show more than minimal astrocytosis, as distinct from the animals in the later groups.

In view of the possible influence of pool size on these findings animals were injected with labelled acetate diluted with up to 5 times the amount of unlabelled acetate and the incorporation into histone determined. The results showed that acetate concentration within this range did not influence histone acetylation significantly (Table II).

Table I. The effect of time on the incorporation of acetyl into brain histones in vivo

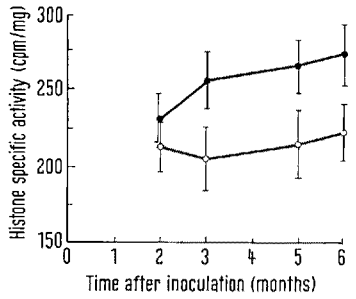
Time (h)	Specific activity (cpm/mg protein)
2	230 ± 20 (4)
4	250 ± 23 (4)
19	290 ± 27 (3)

No. of animals in brackets.

Table II. The effect of acetate concentration on the incorporation of acetyl into brain histone in vivo

Acetate (μg)	Specific activity (cpm/mg protein)[a]	
	Normal	Scrapie
5	227 ± 19 (4)	260 ± 20 (4)
30	208 ± 21 (4)	249 ± 17 (4)

[a] After 2 h incorporation with C^{14} acetate. No. of animals in brackets.



The effect of time after challenge with scrapie on histone acetylation. Each point represents the mean of 4 experiments, standard deviation is indicated by the vertical lines. Period of incorporation with C^{14} acetate 2 h. Controls, open circles; scrapie, filled circles.

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Discussion. Acetylated histone in scrapie mouse brain was increased by about 25% and this appeared before onset of clinical disease. This suggests that an early effect of scrapie infection is a disturbance of the acetylation mechanism. It is believed that this system may form part of a switching mechanism acting on the rate of RNA synthesis and associated reactions; it has also been shown that acetylation is not affected by puromycin which inhibits the incorporation of amino acids into histones and must be subject to a different control mechanism[5]. Similarly a study of acetylation in tobacco mosaic virus protein did not demonstrate any acetyl-amino acid intermediates[10] suggesting that acetate is not activated and transferred by RNA's as are the amino acids. It is believed that the present study has demonstrated an in vivo breakdown of the control mechanism acting on nucleic acid and protein synthesis and it is further suggested that this defect may be confined specifically to the astrocytes. Hyperactivity of the astrocytes constitutes the major cellular abnormality in scrapie and increased acetylhistone formation as well as increased DNA synthesis[11] could be logically attributed to this group of cells. The high specificity of the acetylhistone could then arise as a response to a more general disturbance producing a histone complexing with a specific RNA and obtaining its cellular specificity from the ability of the combined RNA to detect its complementary operator[12]. A similar effect occurs in lymphoid cells which respond in this manner to phytohemagglutinin[6]. Scrapie agent is present in high concentration in lymphoid tissues[13] and further investigations are in progress to examine the possibility of cells in these organs being activated without showing any visible abnormality[14].

Zusammenfassung. Im Gehirn von Mäusen mit Traberkrankheit (Scrapie) war die Azetylierung von Histonen um 25% erhöht. Diese chemische Änderung erschien weitaus früher als klinische Symptome und histologisch-pathologische Veränderungen. Die Beziehung von Azetyl-Histonen zu Deoxyribonukleinsäuren und ihre Verhältnisse zur Aktivierung von spezifischen Nervenzellen werden kurz besprochen.

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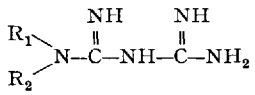
Effect of Biguanides on the Respiration of Tumour Cells

The biguanide derivatives, of general formula



in which R_1 and R_2 can be alkylic, arilic or cycloaliphatic groups, have long been known for several pharmacological activities: hypoglycemic, antiviral, antimalarial, antibacterial and antitumoural. We have synthetized a certain number of biguanide derivatives and we have carried out a series of experiments[1-5] on their antitumoural properties. Many derivatives were able to inhibit in vivo, to the extent of 30–50%, the growth of the Ehrlich ascites tumour in the mouse.

Another characteristic of these compounds is to depress the mitochondrial respiration and the incorporation of phosphate in ADP; these compounds are presumably acting at the level of oxidative phosphorylation[6,7].

Our purpose was to demonstrate whether there could be a connection between the in vitro effect on mitochondria and the antitumoural activity. Thus some biguanide derivatives which are active in vivo [N^1,N^1-dimethylbiguanide hydrochloride (DMB); N^1-benzylbiguanide hydrochloride (BB); N^1-propyl-biguanide hydrochloride (PB); N^1-isopropyl-biguanide hydrochloride (iPB); N^1-butyl-biguanide hydrochloride (BuB); N^1-isobutyl-biguanide hydrochloride (iBuB)] were tested in vitro on the respiration of Ehrlich ascites tumour cells. Another derivative [N^1,N^1-methyl-phenyl-biguanide hydrochloride (MFB)] inactive in vivo was similarly tested in vitro.

In order to show evidence for a selective action of these compounds towards the neoplastic cells, we tested their action in parallel on hepatic cells.

The cellular oxygen uptake was measured by conventional manometric techniques, with or without addition of various biguanides.

The tumour cells were obtained from mice with an 8-day tumour. The hepatic cells were obtained from rats which had been fasting for 48 h in the following way: the liver was perfused through both the lower vena cava and the aorta; the perfusion liquid (NaCl 0.094 M; EDTA 0.0109 M; glucose 0.045 M sodium phosphates buffer pH = 7.4 0.01 M at 37 °C) and blood flowed out through the excised portal vein. The cells, separated by pushing the organ through a thin wire-gauze, were then suspended in Ringer-phosphates (pH 7.4). For each vessel we used

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