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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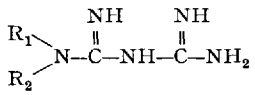
Effect of Biguanides on the Respiration of Tumour Cells

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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2×10^7 cells and glucose (0.01 *M*) as substrate (0.5 ml/vessel). The bath temperature is 37 °C.

The Table shows the doses used, chosen on the base of preliminary tests carried out to establish the more active dose; it also shows the values of percentage inhibition of respiration of tumour and hepatic cells and the corresponding percentage inhibition of tumour development in vivo. The results are also shown as specific activity, in comparison with the DMB activity which is defined as equal to 1.

The biguanide derivatives which have been tested show a slight inhibitory action on the hepatic cells respiration. The inhibitory action is much stronger on the oxygen consumption of the tumour cells; MFB, inactive in vivo, is practically so in vitro.

As a conclusion we can confirm that these biguanide derivatives, which have antitumoural activity in vivo, show an inhibitory action on respiration and that this action is selective toward tumour cells.

Therefore, we could propose the following hypothesis: the antitumoural action of such compounds could be caused by an inhibition of the synthesis of high energy phosphate compounds.

Compound	mg/vessel	Percentage inhibition of O_2 uptake in vitro		Specific activity (tumour cells)	Percentage inhibition of tumour growth in vivo
		Hepatic cells	Tumour cells		
DMB	5	2	72	1.00	30
BB	2	23	91	3.16	49
PB	5	15	85	1.18	47
iPB	8	10	65	0.56	34
BuB	10	12	88	0.61	41
iBuB	7	22	78	0.77	51
MFB	10	7	24	0.17	–

Riassunto. È stato studiato l'effetto di un gruppo di derivati biguanidici sulla respirazione in vitro di cellule tumorali ed epatiche. I composti esaminati sono tutti capaci di inibire, in vario grado, lo sviluppo del tumore ascite di Ehrlich del topo, tranne uno, privo di attività antitumorale in vivo. Tutti i composti mostrano di deprimere lievemente il consumo di ossigeno delle cellule epatiche. I derivati attivi in vivo bloccano quasi completamente la respirazione delle cellule tumorali, mentre il composto inattivo ha un'attività assai inferiore. Si fa l'ipotesi che l'azione antitumorale dei derivati biguanidici si esplichi attraverso una inibizione dei processi respiratori collegati alla formazione di legami altoenergetici.

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A New Antibacterial Agent Produced by *Streptomyces* sp. Ac_6569

A number of streptothricin[1] type of antibiotic, e.g. geomycin[2], racemomycin-O[3], roseothricin[4], streptolin[5] and viomycin[6] have been reported in the literature. A new antibacterial agent, belonging to this group of antibiotics, was isolated from the culture broth of *Streptomyces* sp. Ac_6569, which showed high activity against bacterial and fungal test organisms.

The active material was produced in shake flasks in a medium containing soya peptone, 6 g/l; yeast extract, 2 g/l; KCl, 4 g/l; $(NH_4)_2\ SO_4$, 5 g/l; KH_2PO_4, 0.4 g/l; $CaCO_3$, 0.5 g/l; glucose, 2 g/l and pH was adjusted to 7.2 before sterilization. Elaboration of the antibiotic was measured by assay with *Staphylococcus aureus* and peak titres were usually obtained after 3–4 days incubation at 28 °C.

Isolation and purification of the active material was carried out by adsorption of the active material with Darco G-60 (25 g/l); elution of the active material with 0.1 *N* methanolic HCl, neutralization and concentration of the eluate under reduced pressure and purification by chromatography on a column of Darco G-60; Celite 545 (1:1) using 1% aqueous acetone (v/v) as the eluting agent.

The active material was found to be a tetra-acidic base and isolated in the form of hydrochloride as a pale yellow amorphous material, m.p. 216–218° (with decomposition). Probable molecular formula for the antibiotic was suggested as $C_{23}H_{43}N_9O_8$, 4HCl. No characteristic UV-absorption was observed in aqueous solution. IR-spectrum is indicative of the presence of carbonyl and guanidino type of grouping in the compound. The active material is

Comparative in vitro activity of Ac_6569-sulphate with some of the known antibiotics

Test organism	Minimal inhibitory concentration μg/ml of			
	Ac_6569 sulphate	Kanamycin sulphate	Neomycin sulphate	Streptomycin sulphate
Staphylococcus aureus (sensitive)	3.0	3.0	0.125	3.0
Staphylococcus aureus (resistant)	5.0	3.0	0.25	3.0
Staphylococcus albus	0.5	1.0	0.25	5.0
Bacillus anthracis	8.0	0.5	0.25	2.5
Bacillus subtilis	3.0	1.0	1.0	1.0
Bacillus megaterium	2.5	1.0	1.0	3.0
Proteus vulgaris	12.0	3.0	6.0	10.0
Escherichia coli	0.5	0.5	0.25	0.5
Aerobacter aerogenes	3.0	3.0	1.0	3.0
Pseudomonas aeruginosa	40.0	50.0	20.0	30.0
Salmonella typhosa	6.0	4.0	2.0	15.0
Candida albicans	2.0	–	–	–

–, Not tested.