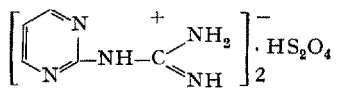
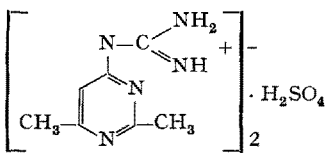
In vitro Polio Virus Inhibition by Two Guanidino-Pyrimidines

Some time ago we demonstrated that guanidine is endowed with a strong inhibiting activity against polio-virus replication *in vitro*[1]. Since all the guanidine derivatives so far examined lack inhibitory action against polio-virus growth, CROWTHER and MELNICK[2] concluded that this antipolio action of guanidine is extremely specific. Our own previous results[3–5] agree well with this conclusion. However, the present experiments show that some substitutions in the guanidine molecule are compatible with a high antipolio activity. In fact two pyrimidine-guanidine synthetized by one of us (A.G.)[6], N-2-guanidino-pyrimidine sulfate (compound E) and N-4-guanidino-2, 6-methyl-pyrimidine sulfate (compound B), have been found to inhibit strongly, *in vitro*, polio-virus multiplication.



N-2-guanidino-pyrimidine sulfate (compound E)



N-4-guanidino-2,6-methyl-pyrimidine sulfate (compound B)

Table I. Inhibitory activity of compounds E and B on the CPE[a] of different viruses

Virus strain	Drug tested	Concentration (γ/ml)	CPU[b] titer 5 days after the inoculum[c]
Polio 1 Brunhenders	–	–	10^8
Polio 1 Brunhenders	E	1333	10
Polio 1 Brunhenders	E	1000	10^2
Polio 1 Brunhenders	E	500	1.2×10^4
Polio 1 Brunhenders	E	250	2×10^6
Polio 1 Brunhenders	E	166	10^8
Polio 1 Brunhenders	B	500	10
Polio 1 Brunhenders	B	333	10^3
Polio 1 Brunhenders	B	166	10^7
Polio 1 Brunhenders	B	111	10^8
Coxsackie B_3	E	–	10^8
Coxsackie B_3	E	1000	10^2
Coxsackie B_3	E	500	10^3
Adenovirus 5	–	–	10^5
Adenovirus 5	E	1000	10^5
Vaccinia virus	–	–	10^7
Vaccinia virus	E	1000	10^7

[a] Cytopathic effect. [b] Cytopathic units. [c] In human amniotic cells (Mascoli).

Table II. Effect of compound E on CPE of guanidine-sensitive, guanidine-resistant and guanidine-dependent polio virus-strains

Virus strain	CPU titer 5 days after inoculum[a]								
	Drug-free medium	In the presence of guanidine HCl (γ/ml)				In the presence of E (γ/ml)			
		12.5	50	100	200	15.6	62.5	250	1.000
Polio 1 S (Brunhenders)	10^8	10^8	10^7	10^5	10^3	10^8	10^8	10^6	10^3
Polio 1 R[b]	10^7	10^8	10^7	10^7	10^7	10^7	10^8	10^7	10^7
Polio 1 D[c]	10^3	10^3	10^7	10^8	10^7	10^5	10^6	10^6	10^7

[a] See Table I. [b] 1 R = 1 S transferred in HeLa cells in the presence of guanidine-HCl: once 1/16,000 and three times 1/4000. [c] 1 D = 1 R transferred 29 times in HeLa cells in the presence of guanidine-HCl 1/4000.

The experiments, performed as previously described[5], have shown that there are several similarities between the antiviral activity of both compounds E and B and guanidine, i.e. (1) Compounds E and B inhibit very effectively polio-virus and Coxsackie B_3 virus, but, like guanidine, they are ineffective against vaccinia and adenovirus 5 (Table I). (2) Either drug-resistance or drug-dependence have been obtained by passing polio-virus in the presence of increasing concentrations of compound E (Table II). (3) Finally, a cross resistance and a cross dependence exist between guanidine and compounds E and B (Table III).

Table III. Effect of guanidine and guanidine-pyrimidines on the CPE of E-dependent polio virus

Virus strain	Drug tested	γ/ml	CPU titer 5 days after inoculum[c]
1 S[a]	–	–	10^8
1 S	Guanidine HCl	200	10^4
1 S	E	500	1.2×10^4
1 S	B	500	10
E 17[b]	–	–	10^4
E 17	Guanidine HCl	200	10^8
E 17	E	500	10^8
E 17	B	500	10^7

[a] 1 S = Polio 1 Brunhenders. [b] E 17 = 1 S transferred in HeLa cells in the presence of compound E: i.e. once 1/20,000; once 1/10,000; once 1/5000; twice 1/2000; three times 1/1000; nine times 1/750. [c] In human amniotic cells (Mascoli).

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[3] B. LODDO, Boll. Soc. Ital. Biol. sperim. *37*, 537 (1961).
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[5] B. LODDO, W. FERRARI, G. BROTZU, and A. SPANEDDA, Boll. Ist. Sierot. Milan *41*, 111 (1962).
[6] The drugs tested were synthesized at the 'Centro Ricerche' of Istituto Chemioterapico Italiano, Milano (Italy).

In conclusion, our results do not support either Melnick's or our own previous assumption that any modification in the guanidine molecule is incompatible with its antiviral activity.

Riassunto. La 2-guanidino-pirimidina solfato e la 4-guanidino-2,6-dimetilpirimidina solfato inibiscono *in vitro* la moltiplicazione del poliovirus e del Coxsackie B_3 ma non quella del virus vaccinico e dell'adenovirus 5. L'analogia di comportamento con la guanidina è documentata oltre che da ciò, dalla esistenza di una resistenza e di una dipendenza crociata. Si dimostra cioè che non tutte le sostituzioni nella molecola della guanidina comportano una perdita dell'attività antivirale, come ritenuto in precedenza.

B. Loddo, A. Garzia, and W. Ferrari

Istituti di Farmacologia, Igiene e Microbiologia dell'Università di Cagliari (Italy), July 17, 1963.

The Specificity of Histones in Chicken Erythrocytes

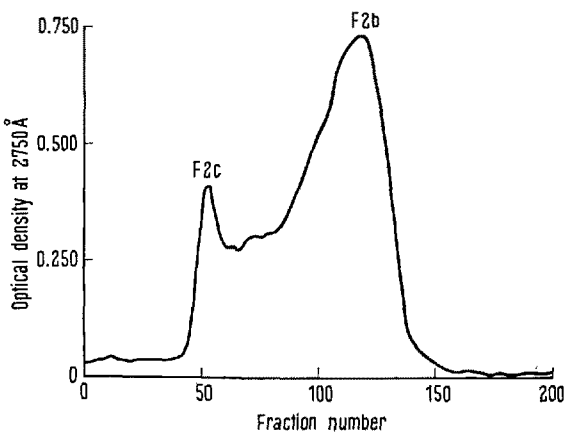
Because of their location in cell nuclei and their ability to interact strongly with deoxyribonucleic acid (DNA), histones may play a key role in the structural organization of DNA and may affect the regulatory functions of DNA. If histones act as gene inhibitors, as was suggested by Stedman and Stedman[1,2], then the histones from different species should show specificity of chemical composition and physicochemical behavior. However, species and cell specificity of histones is controversial; many histones prepared from different tissues of the Vertebrata genus are strikingly similar. Neelin and Butler[3,4] have reported specific elution and starch gel electrophoretic patterns for histones from different tissues of chicken, but gave no analytical data to support this claim of specificity. Stedman and Stedman[1,2] found that, when purified to constant composition, the main and subsidiary histones from the erythrocytes and thymocytes of the fowl differed in their arginine contents. In their more recent paper, Mauritzen and Stedman[5] reported the specificity of amino acid composition of the arginine-rich β-histones from erythrocytes, spleen and liver of domestic fowl. The differences, however, are rather small. To obtain more data, experiments on the isolation and purification of histones from chicken erythrocytes were initiated.

For the present study, fresh blood was collected from decapitated chickens and washed three times with 0.14 *M* NaCl containing 0.01 *M* trisodium citrate. The red cells were sedimented by centrifugation and then hemolyzed by freezing and subsequent thawing. Disrupted erythrocytes were homogenized in a Waring blendor with 0.14 *M* NaCl containing 0.01 *M* trisodium citrate and centrifuged[6]. This washing procedure was repeated several times until the supernatant fluid was clear. The yellowish sediment was then washed with 80% ethanol and extracted with ethanol-HCl and with 0.2 *N* HCl[6,7], yielding fractions F2aF3 and F1F2b, respectively.

The two main fractions, arginine-rich (F2aF3) and lysine-rich (F1F2b), were analyzed by electrophoresis in starch gel[6-8]. A pattern consistent with that described for the similar fraction in mammalian tissues, i.e. two main bands close to the origin (F3), and two bands with high electrophoretic mobility (F2a) resulted from the arginine-rich F2aF3 fraction electrophoresis. Electrophoresis of the lysine-rich group F1F2b revealed the presence of a band absent in mammalian tissue electrophoresis, located between the two sharp zones of very lysine-rich histone F1 on one side and the broader band of N-terminal proline fraction F2b on the other[6,7].

An attempt was made to separate this fraction represented by a band of intermediate electrophoretic mobility in starch gels from the F1 and F2b components. The whole F1F2b mixture was chromatographed on a carboxymethyl cellulose column (Cellex, Calbiochem) using potassium acetate buffer pH 4.2 as eluent[9]. The very lysine-rich fraction F1 was eluted with 0.33 *M* KCl, and the intermediate band emerged from the column together with the F2b fraction, suggesting their close similarity.

Further separation of the intermediate band protein from the F2b histone was achieved by chromatography on Sephadex G 75 (medium grade). Columns 2.6 × 120 cm were filled with the G 75 Sephadex made up in 0.01 *N* HCl. Protein, 200 mg in 2 ml of 0.01 *N* HCl, was applied to the column and HCl of the same normality was used as eluent, and 1.0 ml fractions were collected every 10 min. The separation of the F2b histone into two peaks is shown in the Figure. Fractions No. 42–62 and No. 95–140



Elution pattern for histones of chicken erythrocytes F2b after chromatography on Sephadex G 75 in 0.01 *N* HCl.

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