In brief, the sole basis of Galimov's claim, in connection with his postulated mechanism, that "there are no limitations involving the total concentration of carbon in the medium" is his complete disregard of such limitations, which are inescapable.

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BIOLOGICAL SCIENCES

A Novel Substituted Guanidine with High Activity *in vitro* against Rhinoviruses

THE number and diversity of the viruses which infect the upper respiratory tract, and cause the common cold, virtually preclude the control of this disease by conventional vaccines. From this, apart from symptomatic therapy, the only hope for effective treatment of this most prevalent of diseases is in the development of broad spectrum antiviral drugs. We have developed a substituted guanidine, ICI 65,709 (ref. 1) (Fig. 1) which has good activity *in vitro* against all twenty-five rhinovirus serotypes tested in human embryonic lung cells, and also against certain other picornaviruses.

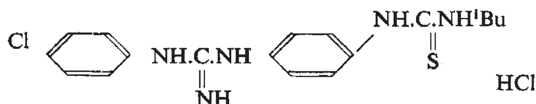

Fig. 1 The structural formula of ICI 65,709; 1-*p*-chlorophenyl-3-(*m* isobutylthioureido) phenyl guanidine hydrochloride.

The method of testing for antiviral activity has been fully described[2,3]. In brief, it consists of infecting tissue-culture cells in 3×0.5-inch tubes with 100 TCD_{50} of test virus, and then adding the test compound in a range of concentrations. After incubation at 33° C for 2 d, the cells are inspected with a low power microscope and the concentration of compound which causes 50% cytotoxicity and that which causes 50% inhibition of virus growth (cytopathic effect) are determined. From these values a tissue-culture 'therapeutic ratio' is calculated for each virus tested.

Table 1 shows that although the range of viruses tested was limited, it is clear that the activity of the compound is not confined to the rhinoviruses. Certain other picornaviruses were sensitive, and there was also some activity against vaccinia, herpes simplex, and the two arboviruses tested. The compound was inactive against equine rhinovirus, respiratory syncytial virus and the 229E strain of coronavirus, and also against influenza or parainfluenza grown in calf kidney cells.

A curious feature of the compound was that its antiviral action was highly dependent on the type of cell in which it is tested. For example, although the growth of rhinovirus type 2 in human embryonic lung cells is strongly inhibited by ICI 65,709, the same virus grown in KB cells, HeLa or primary rhesus monkey kidney cells is completely insensitive to the compound. When rhinoviruses are grown in fragments of human embryonic trachea, 1 μg ml^{-1} ICI 65,709 inhibits growth by approximately 90%.

Table 1 50% Toxic and 50% Antiviral Concentrations of ICI 65,709 Against Various Viruses

Cell type	50% toxic concentration (μg ml^{-1})	Virus	50% antiviral concentration (μg ml^{-1})	Therapeutic ratio *in vitro*
Human embryonic	6	Rhinovirus type		
		6, 5, 35	0.4	15
		33, 3, 44, 40, 1A, 9, 43	0.2	30
		32, 14, 29, 27, 11, 1B, 2	0.1	60
		15, 16, 31, 4, 26, 36, 17	0.05	120
		Equine rhinovirus	Not active	—
		Echovirus type		
		11	0.05	120
		14	0.1	60
		Coxsackie virus type		
		A9	0.4	15
		A21 (Coe)	0.8	7
		B3	0.1	60
		Vaccinia	0.2	30
		Herpes viruses:		
		H. simplex type 1	1.0	6
		H. simplex type 2	1.0	6
		Pseudorabies	Not active	—
		Semliki forest	0.8	7
		Sindbis	0.8	7
Primary calf kidney	12	Influenza		
		A_0	Not active	—
		A_1	Not active	—
		A_2	Not active	—
		Parainfluenza 1	Not active	—
		Respiratory syncytial	Not active	—
		Coronavirus 229E	Not active	—

Studies on the mode of action of ICI 65,709 using rhinovirus type 2 in human embryonic lung cells have shown that the compound does not destroy the rhinovirus virion, nor does it prevent the attachment or penetration of virus particles into the host cell. When the compound is added to virus-infected cells at various times after infection, and the virus yields at the end of the first growth cycle are measured, the compound is seen to exert progressively more inhibition of virus growth the longer it is present on the infected cell. This suggests an effect on a virus synthetic process which begins soon after eclipse and continues throughout the replicative process: possibly virus RNA or protein synthesis. The compound has no inhibitory effect on the RNA or protein synthesis of the host cell at concentrations up to 100 times the 50% antiviral level.

Although ICI 65,709 is a derivative of guanidine, its antiviral action differs from that of guanidine[4] in at least two ways. First, it does not give rise to drug-resistance as does guanidine, and second, its action is not reversed by substances such as ethanolamine, choline, methionine, or lactalbumin hydrolysate[5–8]. Biochemical studies (Kolias, S. I., and Dim-

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mock, N. J., unpublished) also suggest that ICI 65,709 differs in its mode of action from guanidine.

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C-reactive Protein-like Precipitins in Plaice

C-REACTIVE protein (CRP) is an acute phase protein appearing in the serum of man during the febrile stage of infections with pneumococci and various other microorganisms[1,2]. The protein can also be detected in serum and other serous fluids during pathological conditions in which tissue injury, inflammation or carcinoma are involved[3,4]. Acute phase proteins have also been detected in the sera of a number of other vertebrates, including monkeys, rabbits and mice[2,5], but there seem to be few reports of these proteins in lower vertebrates[6].

In the presence of Ca^{2+}, CRP is precipitated from solution by pneumococcal C-substance[1,7] and also by extracts from a number of fungi, invertebrates and plants[8-10]. Following the demonstration that various phosphate monoesters inhibit the CRP-C-polysaccharide precipitation reaction[11], phosphorylcholine has recently been found to be the most active inhibitor of all the compounds so far tested[12].

Using extracts from *Diplococcus pneumoniae*, some fungi and a nematode, *Ascaris lumbricoides*, together with a peptido-polysaccharide isolated from the dermatophyte *Epidermophyton floccosum*, we have found a CRP-like serum component in the plaice (*Pleuronectes platessa* L.), a marine teleost. We describe here specificity studies carried out with the plaice precipitin and discuss the origin and possible biological role of such proteins in poikilotherms.

Fish were caught in shallow water off the coast of Aberdeen and transferred to aerated seawater at 11–14° C in the aquarium. Single serum samples were collected[13] between 1 h and 4 months after the capture of the fish. Human serum containing CRP was obtained from the Brompton Hospital, London, from patients with pulmonary diseases. The presence of CRP in the human samples was established by precipitation with rabbit anti-CRP (Behringwerke). Aqueous extracts of *D. pneumoniae*, *E. floccosum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichophyton mentagrophytes*, *T. rubrum* and *A. lumbricoides* were prepared from culture filtrates and minced worms by methods already described[1,8,14].

Precipitation studies with con A have shown that this reagent reacts with polysaccharides from a variety of fungi including *E. floccosum* and a number of *Aspergillus* and *Trichophyton* species (B. A. B. and J. Pepys, unpublished). A peptido-polysaccharide was isolated from *E. floccosum* by passage of the aqueous extract through a column of Sepharose-con A (Pharmacia) and elution of the adsorbed material with 0.1 M α-methyl-mannopyranoside in physiological saline. After dialysis and lyophilisation, the peptido-polysaccharide was used in precipitation studies.

Forty-four of forty-seven different plaice sera examined (from thirty males and females of approximately 2 to 7 yr and seventeen O-group plaice) precipitated in gel with *E. floccosum* extract. The sera also precipitated with the peptido-polysaccharide and the lines were completely confluent with those formed with culture filtrate extracts from the five fungi examined and extracts from *A. lumbricoides* and *D. pneumoniae*. The precipitin reactions observed when plaice serum and human CRP-containing serum were examined in gel diffusion experiments with some of the extracts are shown (Fig. 1*a* and *b*). As with the plaice serum, the human serum formed a continuous line of precipitation with all of the extracts. When the plaice and human sera were examined side by side in gel with *E. floccosum* peptido-polysaccharide, *E. floccosum* culture filtrate extract and pneumococcus extract, precipitation occurred in front of each of the peripheral wells and the precipitin lines merged with no sign of spurring (Fig. 1*c*).

Our evidence supports the view that the precipitins in the plaice sera are not antibodies. Immersion of the gels in 5% sodium citrate or 0.1 M EDTA in physiological saline caused the disappearance, within 30 min, of the lines between the plaice serum and human CRP and the pneumococcus, fungal and *Ascaris* extracts. In contrast, citrate or EDTA did not affect the precipitin reaction observed between *Vibrio anguillarum* extract and serum from a plaice previously immunised with this antigen[13]. Immunoelectrophoresis of whole plaice serum in Ionagar at *p*H 8.6 followed by development against *E. floccosum* culture filtrate extract or peptido-polysaccharide revealed a precipitin line in the α_2-region, and antibody activity was associated with an immunoglobulin migrating in the β-region. The plaice and human CRP precipitin lines were dissolved within 2 h after immersion of the gels in 0.001 M phosphorylcholine in physiological saline. The lines were not soluble in 0.002 M solutions of choline chloride, α-D-glycerophosphate, AMP, CMP, UMP, glucose-1-phosphate, glucose-6-phosphate or in phosphate-buffered saline. Precipitation of plaice serum with *E. floccosum* peptido-polysaccharide and inhibition of this reaction with phosphorylcholine was confirmed with quantitative studies. In a typical experiment (Table 1), 200 μl of plaice serum produced maximum precipitation (2.6 μg of protein N) with 1,086 μg of *E. floccosum* peptido-polysaccharide. Using these quantities of reagents, inhibition studies revealed that precipitation could be partially inhibited (76%) by 1 μmol of phosphorylcholine and completely inhibited by 10 μmol.

Table 1 Precipitation of *Epidermophyton floccosum* Peptidopolysaccharide by Plaice Serum

Peptido-polysaccharide used (μg)	N in precipitate (μg)
108.6	0.42
217.2	0.76
543.0	1.90
760.2	2.30
1,086.0	2.55
1,303.2	2.42

Quantitative precipitin analysis was carried out using a microprecipitin technique and ninhydrin procedure for nitrogen determination[21]. Volume of serum per tube, 200 μl. Total volume, 350 μl.

The finding of CRP-like precipitins in the sera of apparently healthy male, female and young plaice, together with the fact that these precipitins were detected in almost all of the fish examined, suggests that this protein may be a normal component of plaice serum and not arise, as in higher vertebrates, only after infection or trauma. Some of the plaice examined had been in the aquarium for 4 months and were feeding

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