while the concentration effects for potassium and sodium were not statistically significant. The overall combined effects of salt and concentration (salt × concentration interaction) on the frequency of contraction was also significant ($p < 0.001$), and significant pairwise salt × concentration interactions were found for lithium and rubidium ($p < 0.001$), lithium and potassium ($p < 0.025$) and lithium and sodium ($p < 0.005$). The salt × concentration interaction for rubidium and sodium ($F = 2.3$, $df = 10/176$) just failed to attain significance at the 5% level, while the interactions for rubidium and potassium and for sodium and potassium were not statistically significant.

Discussion. Our findings show that addition of lithium or rubidium to sea water influenced the frequency of contraction of jellyfish. It is noteworthy that the changes in the frequency of contraction occurred without noticeable changes in the rate, evenness and uniformity of individual contractions. These observations suggest that lithium and rubidium affected mainly the rate of firing of the pacemakers in the marginal ganglia responsible for the rhythm of swimming movement[10–12]. It is of interest therefore to consider the actions of lithium and rubidium on ganglionic transmission.

The most consistent effect of lithium on ganglia is impairment of transmission, due primarily to lithium-induced depolarization of postsynaptic membranes[13–17]. While this action may be able to account for the decline in the frequency of contraction seen at lithium concentrations above 6 mmoles/l, it probably cannot explain the rise in the frequency of contraction seen at lower lithium concentrations. Thus, other effects of lithium on synaptic transmission may be involved in the effects of low concentrations of lithium on the frequency of contraction of jellyfish.

Rubidium is thought to act on ganglionic transmission by prolonging presynaptic action potentials, perhaps due to rubidium-induced hyperpolarization of synaptic membranes[8]. Prolongation of action potentials by rubidium could enhance neurotransmission, increase the rate of firing of pacemakers, and increase the frequency of contraction of jellyfish.

Electrophysiological studies clearly are needed to determine whether these explanations for the effects of lithium and rubidium on the swimming rhythm of jellyfish are correct.

In conclusion, a word should be said on the notion that lithium and rubidium can be expected to have opposite effects on biological processes[7,8,18]. We agree with those who consider there to be as yet too little evidence to support such an expectation in general[19]. It is to be noted, nevertheless, that lithium and rubidium, at concentrations between 6 and 30 mmoles/l, had opposite effects on the frequency of contraction of jellyfish in the present experiment. This observation suggests that studies on phylogenetically low animals, such as jellyfish, may be of use to determine relationships between neurophysiological and behavioural actions of lithium and rubidium.

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Hydrogen bonding by the sulphydryl group of glutathione

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Summary. The occurrence of hydrogen bond in the sulphydryl group of glutathione was investigated by means of Raman Spectroscopy. Evidence is obtained that SH group is free and H-bonding does not occur.

Hydrogen-bonding properties of OH and NH groups are well documented and established; on the contrary, the existence of SH--X bond is still controversial, at least in biological thiols[1–3]. In glutathione (GSH) the formation of intramolecular H-bond, involving the sulphydryl group, has been suggested[4–6] for explaining the reactive behaviour of -SH (for instance, the effect of hydrogen bond-breaking reagents, such as urea, on the reaction rate with AG^+ or nitroprusside, or the formation of a thiazolidine ring in strongly acidic solutions), but direct evidence is lacking.

The frequency of SH stretching vibration ν(SH), shifting to lower wavenumbers upon H-bond formation, is a sensitive test of the occurrence of hydrogen bonding: it is therefore worthwile to examine carefully by vibrational (Raman) spectroscopy the ν(SH) region of GSH. Raman spectra were taken with a Jarrell-Ash Raman Spectrometer, using an Ar^+ laser (4880 Å line) as excitation source and with a spectral resolution of 1.5 cm^{-1}.

In the solid state, the ν(SH) frequency is surprisingly low (2530 cm^{-1}) as compared with those of other simple thiols (see table 1), and such a value suggest, intra- or intermolecular H-bond. X-ray structure of GSH[7], however, does

not show interatomic S--O distances shorter than the sum of the Van der Waals radii, so that the low value of the Raman frequency has to be attributed only to the effect of the crystalline environment.

Table 1. SH frequency of thiols in the solid state

Thiol	SH (cm^{-1})
L-cysteine	2545
L-cysteine HCl	2570
L-cysteine-methylester	2565
Dithiothreitol	2570 (unresolved doublet)
	2582
	2588
Glutathione	2530

Table 2. SH frequency of thiols in solution

Thiol	Solvent*	SH (cm^{-1})
CH_3SH	CCl_4	2586
C_2H_5SH	CCl_4	2578
$C_6H_5CH_2SH$	CCl_4	2580
$C_2H_5O(CH_2)_2SH$	CCl_4	2585
$C_2H_5COOCH_2SH$	CCl_4	2582
$NH_2(CH_2)_2SH$	H_2O	2578
$SH-CH_2-(CHOH)_2CH_2SH$	H_2O	2588
$COOHCH_2SH$	H_2O	2580
$COOH-CH(NH_2)CH_2SH$	H_2O	2583
GSH	H_2O	2584

* Data in CCl_4 from Mori[8], data in H_2O this work.

On the other hand, in aqueous solution, the sulphydryl stretching vibration of GSH occurs at higher wavenumbers, 2584 cm^{-1}, and it is independent from pH. This frequency compares well with those of simple thiols, both in water and in CCl_4[8], as shown in table 2. The substantial identity of ν(SH) frequency for very different molecules in both polar and non-polar solvents (at concentration below 0.5 M) excludes a large occurrence of either intra- or intermolecular (solute-solute and solute solvent) hydrogen bonding. Moreover, in all thiols examined, the shape of the ν(SH) Raman band is very similar: it is an essentially symmetric band with a small tail at low frequency side and a typical width of about 25 cm^{-1} in water at room temperature.

All these facts clearly suggest that in GSH the sulphydryl group is free and its reactive behaviour is not influenced by specific H-bonding, but -more probably - by the state of nearby reactive groups.

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Mechanisms of heterotypic immunity against canine distemper[1]

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Summary. Hep-2 cells infected with measles virus (MV) for as short as 6 h became refractory to superinfection with canine distemper virus (CDV) but not to vesicular stomatitis virus (VSV). The exact mechanism of such interference is unknown but probably occurs after virus attachment and penetration. These results verify the suggestion that virus interference may be a mechanism of heterotypic protection against canine distemper.

The application of heterotypic vaccination to canine medicine has been exemplified by the use of measles virus (MV) for vaccination against canine distemper (CDV). Even though CDV antibodies do not neutralize the MV, other cross-reacting antigens of MV allows pups to be sensitized by MV in the presence of anti-CDV maternal antibody[2,3]. Although the principle of this heterotypic immunity remains obscure, several mechanisms have been proposed to account for this phenomenon. These include an induction of a cross reacting antibody response[4,5], delayed type hypersensitivity or cell mediated immunity[6], heterologous viral interference[7] and interferon[7]. Thus far, none of these suggestions has been verified. Supposedly different mechanisms occur at different times post-exposure to measles virus. Thus, humoral and cellular immune response would probably function at times later than 1 week post-vaccination whereas interference would occur earlier. Recently, we have shown that MV can replicate in both canine lymphocytes and macrophages[8] suggesting that blockage at the leukocyte level is possible and could be effective against CDV infection. The present report provides further evidence that MV can interfere with CDV and that interference may be the mayor mechanism of heterotypic immunity against canine distemper at least at early stages of infection.

Materials and methods. Hep-2 cells were grown in 60 mm tissue culture plates (Corning No.25010) in Eagle's minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). Confluent cultures were infected at a multiplicity of infection (MOI) of 1 with MV (Edmonston) or UV-inactivated MV (UVMV). Following a 1-h adsorption period the unadsorbed virus was removed and fresh MEM was added. At different times post-infection (0 h or 6 h) the cultures were superinfected with various concentrations of CDV. The number of CDV plaques were evaluated 24-36 h post-infection[9] and the amount of infectious CDV or MV released into the culture fluid 72-96 h later was determined by a plaque assay[9] on Hep-2 and Vero cells for CDV and MV respectively. To prevent interference by CDV when MV titres were being conducted, anti-CDV antibody was included in the plaque assay. Controls included Hep-2 cells infected with MV followed by vesicular stomatitis (VSV) to ensure that there was no nonspecific interference due to interferon.

To determine whether interference was due to interferon, the supernatant fluid from MV infected cultures were